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JOURNAL OF LEUKOCYTE BIOLOGY

Program and Abstracts of the
TWENTY-FIFTH NATIONAL MEETING
of
THE SOCIETY FOR LEUKOCYTE BIOLOGY
Washington Hilton
Washington, DC
October 27-30, 1988

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An Official Publication of the Society for Leukocyte Biology, incorporated as the Reticuloendothelial Society

This journal will consider for publication manuscripts of original investigations focusing on the origins, the developmental biology and the functions of granulocytes, lymphocytes and mononuclear phagocytes. These reports include full length papers on original research, short communications of new discoveries and invited reviews.

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TWENTY-FIFTH MEETING OF THE SOCIETY FOR LEUKOCYTE BIOLOGY

The Twenty-Fifth Meeting of the Society for Leukocyte Biology was convened this year at the Washington Hilton and Towers Hotel, Washington D.C. between October 27-30th. The meeting comprised three plenary sessions, eight minisymposia and three poster sessions as well as a premeeting discussion workshop on the problems and successes in the quantitative measurement of lipid mediators, wet workshops on spectrofluorimetry and flow cytometry and club meetings focussing attention on topics ranging from aging and the immune response to neutrophil proteases in inflammation.

The scientific programme commenced with the Keynote Address that was delivered by Dr. Philippa Marrack on the subject of the T-cell repertoire. Particular attention was directed towards the issues of control of the T-cell repertoire by several mechanisms including recombination of germ line gene elements, as well as the events that occur in the thymus that lead to the elimination of self-reactive T-cells, and the selection of T-cells bearing receptors that can recognize antigen in the context of self-MHC.

The first of the three plenary sessions entitled "Phospholipids and Cell Activation" focussed on the elicitation of inflammatory cell function. Dr Alan Aderem discussed recent findings from his laboratory on the mechanism of priming of arachidonic acid release from macrophages by bacterial lipoppolysaccharide (LPS). Priming by LPS not only enhances the release of arachidonate in response to challenge with zymosan particles and tetradecanoyl phorbol acetate (TPA), but also induces a competence state that subsequently allows latex particles to trigger arachidonate release, a feature that is not associated upon application of this stimulus to unprimed cells. A prominent feature of the LPS-induced priming response is the myristoylation of a 68 kDa membrane protein. Dr Aderem speculated that the myristoylation may target the 68 kDa protein to the plasma membrane. The 68 kDa protein is also phosphorylated by protein kinase C. This latter event may be important in its release back into the cytoplasm. Unlike LPS, TPA does not induce myristoylation of the 68 kDa protein, but it does promote its phosphorylation. Importantly, evidence was also presented that

myristoylation also accompanies another priming phenomenon, namely that induces by IFNγ. However, under these conditions, the myristoylated protein was clearly different from that myristoylated in response to LPS.

Dr Christina Leslie presented recent findings on the properties of an arachidonyl specific phospholipase A_2 enzyme of the mouse macrophage cell line RAW 264.7. This enzyme has been purified to homogeneity and exhibits a relatively high molecular weight (60 kDa) compared to other phospholipase A_2 enzymes. The enzyme plays a pivotal role not only in the liberation of the arachidonic acid that is utilized in the synthesis of icosanoids, but also in the synthesis of the bioactive ether phospholipid, platelet-activating factor. The phospholipase is largely located in the cytoplasmic compartment of resting cells but becomes associated with a membrane fraction in the presence of Ca^{2+}. A curious and unexpected feature of the phospholipase A_2 is its similarity in many respects to other calcium-phospholipid binding proteins including protein kinase C, such as its requirement for Ca^{2+} and in particular the stimulation of its activity by acidic phospholipids such as phosphatidyl serine and diacylglycerol.

Other presentations focussed on the generation of the second messengers that convey information from the cell surface to the various internal effector mechanisms. 1,4,5-inositol trisphosphate([1,4,5 IP_3]) and diacylglycerol have been ubiquitously recognized as a important second messengers. Dr Charles Rock discussed the biochemistry of a membrane associated phospholipase C that exhibits specificity for phosphatidylinositol 4,5-bisphosphate to yield [1,4,5 IP_3] and diacylglycerol. Dr Rock suggested that phosphatidic acid, produced by the phosphorylation of diacylglycerol, may play an important regulatory role in the control of phospholipase C activity. This observation may in part explain previous suggestions that phosphatidic acid could behave as a calcium ionophore. The link between the cell surface and the phospholipase C is frequently accomplished by G-proteins. This issue was discussed by Dr. Shamshad Cockcroft with reference to her studies on the role of G-proteins in ATP and formyl-peptide induced ß-glucuronidase secretion by neutrophils and differentiated HL-60 cells.

The second plenary session was devoted to a discussion of the involvement of mononuclear phagocytes in retroviral diseases, with a timely and appropriate emphasis on interactions between the human immunodeficiency virus (HIV) and macrophages. Until recently, the predominent host cell implicated in the replication of HIV was the CD4 positive T-helper cell. Macrophage-HIV interactions and the role of the macrophage in the replication of the virus has only emerged during the past 9-12 months. Dr Howard Gendelman reviewed the biology of HIV including its mechanism of interaction with T-cells, and the replication. Using in situ hybridization with an HIV cDNA probe, combined with immunocytochemistry with anti-lysozyme antibodies (to identify macrophages), macrophage rich areas of the brain were shown to harbour abundent amounts of the virus. At the electron microscope level, HIV virions were clearly demonstrable within macrophages. Immensely significant were the findings that HIV could infect macrophages in the presence of GM-CSF, and the fact that when infected T-cells were grown in the presence of infected macrophages, the virus appeared to gradually adapt to itself to become competent to infect the cocultivated mononuclear phagocytes. How HIV is able to infect macrophages is not known, but it appears to take place in a CD4 independent fashion since soluble CD4, while totally blocking virus replication in T-cells, was only partially effective at blocking HIV replication in macrophages.

The third and final plenary session was devoted to recent advances in the biochemistry and molecular biology of oxidase activation in phagocytic cells. Dr Bernard Babior discussed the mechanism of translocation of the oxidase from the cytoplasm to the plasma membrane during neutrophil activation, as well as the role of protein phosphorylation in the formation of the active ternary complex that contains the oxidase. The role and structure of the G-proteins involved in coupling the FMLP receptor to phospholipase C during the activation of neutrophils was discussed by Dr Gary Bokoch, while Dr Al Jesaitis reviewed recent work from his laboratory that has led to the purification and characterization of the subunit structure of the oxidase and the deduction of the amino acid sequence from gene cloning. The regulation of cytochrome b gene expression during mononuclear phagocyte

development and differentiation was discussed by Dr. Peter Newburger. As monocytes differentiate into macrophages in vitro, the expression of the heavy chain gene begins to decline. However, the deficit can be restored by exposure of macrophages to IFNγ. Expression of the oxidase light chain gene however, is not regulated to the same extent as that of the heavy chain gene. Of immense clinical significance were the observations that treatment of monocytes from individuals with X-linked chronic granulomatous disease (CGD) with IFNγ not only up-regulated cytochrome b gene expression, but also functionally restored the ability of monocytes and neutrophils from these individuals to generate reactive oxygen free radicals. These important observations have now formed the basis of a clinical trail of the effects of IFNγ in CGD. In one reported case, a single injection of IFNγ reconstituted a respiratory burst with effects that persisted for up to 20-30 days.

Eight minisymposia addressed issues that included the biochemistry of neutrophil activation and priming, inflammatory mediators and cytokine networks, mechanisms of gene expression during leukocyte development and differentiation, and mechanisms of tumor, parasite, and microbial elimination and destruction. Since four minisymposia were held concurrently on each of two afternoon, it is not possible to give a comprehensive summary of each session.

The spectrum and role of cell surface gangliosides as primary low affinity receptors or secondary binding sites for an array of growth factors, cytokines and bacterial toxins was comprehensively reviewed by Dr. John Ryan in the minisymposium on "Inflammatory Mediators". Dr Stephen Russell, chairing the minsymposium on "Mechanisms of Cellular Cytotoxicity" initially reviewed recent work conducted in his laboratory on the regulation of the activated macrophage phenotype by IFNγ and triggering stimuli such as LPS and double-stranded polyribonucleotides, and on the dynamics of expression of two unique markers of the primed and activated states. Drawing on other work by Dr. Luigi Varesio, Dr. Russell speculated that under certain conditions, notably with the C57/Black mouse, macrophage tumoricidal activity can be elaborated in response to IFNγ alone. A prominent feature of this response is the induction of the enzyme indoleamine-2,3-dioxygenase which is involved in the formation of picolinic

acid by tryptophan degradation. Picolinic acid itself, when added in millimolar concentrations to macrophages, induces the cytolytic phenotype. However, the relationship between picolinic acid and the triggering of cytolysis by other agents such as LPS is not as yet apparent. A possibility is that precursor rRNA molecules which are accumulated during macrophage activation and which, perhaps as a consequence of elements of internal double-strandedness, may enhance cytolytic activity in much the same way that polyribonucleotides have been suggested to do so. In the search for macrophage phenotypic markers, Dr. Paul Johnston described recent studies from his laboratory on p120, a macrophage protein marker of the cytolytic phenotype. Using a monospecific antiserum, evidence was presented that while p120 itself is not implicated in the expression of cytolytic activity, it is nevertheless a useful phenotypic marker of activated mouse peritoneal macrophages.

Collectively, papers presented at several minisymposia, focussed attention on the regulation of cytokine production by mononuclear phagocytes, or on the effects of cytokines on mononuclear phagocyte function. With a broader appreciation of the breadth of cytokines that are produced and act on mononuclear phagocytes, it is becoming clearer cytokine biology is considerably more complex that perhaps at first suspected in that many cytokines have overlapping activities, while single cytokine species frequently express, in a dose dependent fashion, both agonist and antagonist activities. Several papers discussed the autocrine-paracrine regulatory functions of macrophage derived molecules such as the role of 1,25-dihydroxyvitamin D_3 in the differentiation of, and expression of protooncogenes by, myeloid precursor cells.

The Presidential and Young Investigator Awards Competition have formed an important element of the Leukocyte Biology Meeting for several years. Four contenders for each award presented work ranging from the genetics of aquired immunity to mycobacteria, to the role of neutrophils in experimental myocardial ischaemia. Molecular biological technology has led to major advances in our understanding of the mononuclear phagocyte system and this trend was reflected in the selection of the awardees. The Presidential Award was presented to Dr. Sarah Sporn, University of North

Carolina, Chapel Hill, for her work on the cloning, sequencing and analysis of adherence specific cDNA's derived by subtractive hybridization using a human monocyte cDNA library. The Young Investigator Award went to Dr. Karen MacNaul, Merck, Sharp and Dohme Research Laboratories, Rahway, for investigations into the expression of transcripts for Il-1 and TNF by synoviocytes and monocytes using in situ hybridization as the detecting system. The awards were presented to the winners at the Banquet which traditionally is the high spot of the social calender of the Meeting. The prestigious Maria T. Bonazinga Award for outstanding contributions to the field of Leukocyte Biology was presented to Dr. Marco Baggiolini, University of Bern who delighted all those present at the Banquet with a witty dialogue (complete with "slides") of his research experiences spanning three decades.

All in all, the 25th Meeting of the Society for Leukocyte Biology presented a Scientific Program that covered new ground (e.g. retroviral-macrophage interactions) as well as ongoing, more traditional areas of interest in leukocyte biology. Furthermore, the meeting was well attended, not only by scientists from the U.S.A., but also (perhaps as a consequence of its east coast location) by many of our colleagues from Europe. I am sure we will all look forward to the 26th Meeting of the Society for Leukocyte Biology on Marco Island, Florida, next October.

David W. H. Riches. Ph.D.
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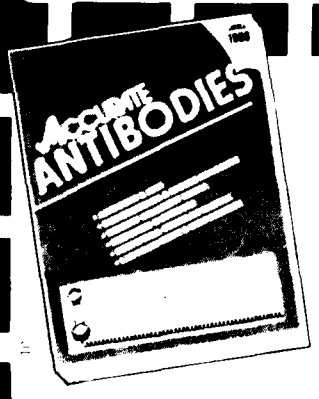

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25th National Meeting of the Society for Leukocyte Biology*	Washington, D.C., Oct. 27–30, 1988

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Twenty-Fifth National Meeting of the Society for Leukocyte Biology

October 27-30, 1988

Washington Hilton, Washington, DC

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RETICULOENDOTHELIAL SOCIETY AWARDS

PRESIDENTIAL AWARD

President Carleton Stewart is pleased to announce that the first place winner for the Presidential Award of the RES Society this year will receive $600 and a plaque. This will be given for the best student paper presented at the Twenty-Fifth National Meeting of the Society for Leukocyte Biology. The second place award will be $300 and a plaque. Competition for this award will include all "candidates in training" (predoctoral and postdoctoral) with a maximum of two years of postdoctoral work.

The first place award is funded by the Office of Naval Research and the second prize by the Annie R. Beasley Memorial Fund. Winners will be announced at the Banquet, Saturday, October 29, 1988.

The winners of the 1987 awards were:

First place:
Chaim O. Jacob
Stanford University
Stanford, CA

Second place:
Eugene Roussel
Manitoba Institute of Cell Biology
Winnipeg, Canada

YOUNG INVESTIGATOR AWARD

This competition is open to all investigators under 36 years of age. The award is $600 and a plaque. The winner for 1987 was:

Gary M. Bokoch
Research Institute of Scripps Clinic
La Jolla, CA

1988 AWARD FINALISTS

Presidential Award:

Ellen Buschman, Montreal General Hospital Research Institute, Montreal,Canada
Diane Hamelin-Bourassa, Montreal General Hospital Research Institute, Montreal, Canada
Sarah Sporn, University of North Carolina, Chapel Hill, NC
Linda Vespa, Ohio State University, Columbus, OH

Young Investigator Award:

Ezio Bonvini, US-FDA, Bethesda, MD
Ronald G. Collman, University of Pennsylvania Medical Center, Philadelphia, PA
William J. Dreyer, Texas Children's Clinical Care, Houston, TX
Karen L. MacNaul, Merck Sharp & Dohme Research Laboratories, Rahway, NJ

STUDENT TRAVEL AWARDS

Student Travel Awards were made based on scientific merit and financial need.

Stephen Apfelroth, SUNY Health Science Center, Brooklyn, NY
Jane A. Armstrong, SUNY Health Science Center, Brooklyn, NY
Andrew Beavis, Rutgers University, Piscataway, NJ
Ellen Buschman, Montreal General Hospital Research Institute, Montreal, Canada
Anthea Dokidis, Rutgers University, Piscataway, NJ
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RES RESEARCH AWARD

The Society for Leukocyte Biology will present the Marie T. Bonazinga Annual Research Award at the Twenty-Fifth National Meeting. This award is sponsored by the Accurate Chemical and Scientific Corporation and is to be presented to a member of the Society who has demonstrated excellence in research. Presentation of the $2,000 award will be made at the banquet on Saturday, October 29, 1988.

The winner of the 1987 award was:

Joost J. Oppenheim
Molecular Immunoregulation
BRMP, FCRF-NCI,
Frederick, MD 21701

The 1988 winner is Marco Baggiolini (see Awards Session, Saturday, October 29, 1988).

HONORARY LIFE MEMBER

Each year, members are considered for election to Honorary Life Membership as a tribute to their contributions to the knowledge of the reticuloendothelial system and their distinguished service to the Society. The recipient in 1987 was:

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TWENTY-FIFTH NATIONAL MEETING OF THE SOCIETY FOR LEUKOCYTE BIOLOGY (A RETICULOENDOTHELIAL SOCIETY)

Washington Hilton, Washington, DC
October 27-30, 1988

Thurs, Oct 27, 1988

		Room
1:00 PM-6:00 PM	REGISTRATION	Concourse Corridor
10:00 AM-4:00 PM	RES COUNCIL MEETING	Adams Room
1:00 PM-5:00 PM	PREMEETING DISCUSSION WORKSHOP: **Problems and Success in the Quantitative Measurement of Lipid Mediators**	Lincoln West
	Chaired by: Robert C. Murphy, University of Colorado, Denver	
1:00-1:15 PM	1. **Overview**: Robert C. Murphy	
1:15-2:15 PM	2. **Radioimmunoassays**, Frank A. Fitzpatrick, University of Colorado, Denver	
2:15-3:15 PM	3. **Enzyme Linked Immunoassays**, Jacques Maclouf, Paris, France	
3:15-3:30 PM	BREAK	
3:30-4:30 PM	4. **Mass Spectrometry-Based Analyses**, Keith L. Clay, University of Colorado, Denver	
4:30-5:00 PM	5. **Summary/Discussion**, Robert C. Murphy	
7:15 PM-8:30 PM	OPENING PROGRAM	Jefferson
GREETINGS	Dr. Carleton C. Stewart, President, RES Society	
WELCOME AND ANNOUNCEMENTS		
INTRODUCTION OF SPEAKER -	Peter M. Henson, Chair, Scientific Program Committee	
7:30 PM	KEYNOTE ADDRESS: **The T-Cell Repertoire**	
	Philippa Marrack, National Jewish Center for Immunology and Respiratory Medicine, Denver	
8:30 PM	RECEPTION	Lincoln/Monroe

Fri, Oct 28, 1988

7:30 AM-6:00 PM	REGISTRATION	Concourse Corridor
7:30 AM-8:30 AM	CONTINENTAL BREAKFAST	Concourse Corridor
7:30 AM-9:30 AM	POSTER SESSION I, Papers 1-38	Thoroughbred and Hemisphere
	Granulocyte Activation, Papers 1-14	
	Host Resistance to Tumors, Papers 15-23	
	Host Resistance to Infection, Papers 24-38	
9:30 AM-12:30 PM	PLENARY SESSION I: **Phospholipids and Cell Activation**	International Ballroom East
	Chaired by: Peter M. Henson, National Jewish Center for Immunology and Respiratory Medicine, Denver	
	1. **Properties of an Arachidonoyl Hydrolyzing Phospholipase A_2 from Macrophages**, Christina Leslie, National Jewish Center for Immunology and Respiratory Medicine, Denver	
	2. **Protein Myristylation as an Intermediate Step in Regulation of Macrophage Function**, Alan Aderem, Rockefeller University, NY	
	3. **Phosphoinositol for 5-biphosphate Phospholipase C**, Charles O. Rock, St. Jude's Childrens Research Hospital, Memphis	
	4. **G-proteins in Phospholipase C in Neutrophil Activation**, Shamshad Cockroft, University College London	
10:30 AM-10:45 AM	COFFEE	Military
12:30 PM-2:00 PM	LUNCH BREAK	
2:00 PM-4:00 PM	RES AWARDS COMPETITION, Papers 39-46	International Ballroom East
	Chaired by: Phil Davies, Chair, Awards Committee	
3:45 PM-4:15 PM	COFFEE available	Concourse Corridor

		Room
4:00 PM-6:00 PM	MINISYMPOSIUM 1, papers 47-52 **Molecular Mechanisms Regulating Leukocyte Development** Chair: Robert Strunk, Washington University Medical Center	Jefferson West
	MINISYMPOSIUM II, papers, 53-58 **Mechanisms of Granulocyte Activation** Chair: Richard B. Johnston, Jr., University of Pennsylvania	Jefferson East
	MINISYMPOSIUM III, papers, 59-64 **Inflammatory Mediators** Chair: John L. Ryan, Yale University School of Medicine	Lincoln West
	MINISYMPOSIUM IV, papers, 65-70 **Phospholipids and Stimulus Transduction Processes** Chair: Filippo Rossi, Istituto di Patologia Generale Universita degli Studi di Verona	Lincoln East
6:00 PM-7:00 PM	BUSINESS MEETING	Lincoln West
Sat, Oct 29, 1988		
7:00 AM	EDITORIAL BREAKFAST	Georgetown East
7:30 AM-12:00 PM	REGISTRATION	Concourse Corridor
7:30 AM-8:30 AM	CONTINENTAL BREAKFAST	Concourse Corridor
7:30 AM-9:30 AM	POSTER SESSION II, papers 71-119	Thoroughbred & Hemisphere
	Cellular Differentiation and Maturation, papers 71-89 Macrophage as Effector Cells, papers 90-97 Cellular Collaboration, papers 98-106 Cytokines, papers 107-119	
9:30 AM-12:30 PM	Plenary Session II: **Mononuclear Phagocytes and Retroviral Disease** Chaired by: Monte S. Meltzer, Walter Reed Army Institute of Research, Washington, DC	Lincoln East & Monroe
	1. **HIV-Macrophage Interactions**, Howard E. Gendelman, Columbia University College Physicians and Surgeons, New York 2. **Macrophages and the Biology of Lentiviruses**, Opendra Narayan, The Johns Hopkins Hospital, Baltimore 3. **Changes in Epidermal Langerhans Cells During HIV Infection**, Georg Stingl, University of Vienna Medical School, Vienna 4. **Macrophages as Target Cells for HIV**, Michael S. McGrath, San Francisco General Hospital, San Francisco	
10:30 AM-10:45 AM	COFFEE	Military Room
	FREE AFTERNOON	
4:00 PM-6:00 PM	MINISYMPOSIUM V, papers 120-125 **Anti-Microbial and Anti-Parasitic Mechanisms** Chair: Emil Skamene, McGill University, Montreal	Lincoln West
	MINISYMPOSIUM VI, papers 126-131 **Cytokine: Effector Cell Collaboration** Chair: Robert D. Schreiber, Washington University School of Medicine	Lincoln East

MINISYMPOSIUM VII, papers 132-137 — Monroe West
Mechanisms of Cellular Cytotoxicity
Chair: Stephen W. Russell, Kansas University Medical Center

MINISYMPOSIUM VIII, papers 138-143 — Monroe East
Cellular Interactions in Inflammation
Chair: Stephen J. Haskill, University of North Carolina

6:30 PM-7:30 PM RECEPTION — Jefferson
7:30 PM-9:30 PM BANQUET — Lincoln and Monroe Rooms
Marco Baggiolini Awardee, University of Bern, Switzerland
THE MARIE T BONAZINGA ANNUAL RESEARCH AWARD OF THE RES SOCIETY

Sun, Oct 30, 1988

7:30 AM-12:00 PM REGISTRATION — Concourse Corridor
7:30 AM-8:30 AM CONTINENTAL BREAKFAST — Concourse Corridor
7:30 AM-9:30 AM POSTER SESSION III, papers 144-192 — Thoroughbred & Hemisphere

Macrophage Activation and Regulation, papers 144-167
Lymphocytes and NK Cells; papers 168-176, 192
Immunopathological Consequences of Host Defense, papers 177-183
Immunopathology, papers 184-191

9:30 AM-12:30 PM PLENARY SESSION III: **Neutrophils and Oxidase Activation** — Lincoln East & Monroe
Chaired by: Bernard M. Babior, Research Institute of Scripps Clinic, La Jolla

1. **Protein Phosphorylation and the Activation of the Respiratory Burst Oxidase in Human Neutrophils**, Bernard M. Babior
2. **Clues to the Function of Human Neutrophil Cytochrome b from its Purification and Structural Characterization**, Algirdas J. Jesaitis, Research Institute of Scripps Clinic, La Jolla
3. **Molecular Genetics of the Superoxide-Generating System of Neutrophils**, Stewart H. Orkin, Children's Hospital, Boston
4. **Regulation of the Cytochrome b Genes in Phagocytes**, Peter E. Newburger, University of Massachusetts, Worcester
5. **The Role of GTP-Binding Protein in Neutrophil Signal Transduction**, Gary M. Bokoch, Research Institute of Scripps Clinic, La Jolla
6. **Studies on Chronic Granulomatous Disease Using the Cell-Free Oxidase Activation System**, John T. Curnutte, Research Institute of Scripps Clinic, La Jolla

10:30 AM-10:45 AM COFFEE — Military Room

PLENARY SESSION I

Friday, October 28th

International Ballroom East

Phospholipids and Cell Activation

Peter M. Henson presiding

9:30 AM
Introduction: Peter M. Henson, National Jewish Center for Immunology and Respiratory Medicine, Denver

9:40 AM
Christina Leslie, National Jewish Center for Immunology and Respiratory Medicine, Denver
PROPERTIES OF AN ARACHIDONOYL HYDROLYZING PHOSPHOLIPASE A_2 FROM MACROPHAGES

10:20 AM
Alan Aderem, Rockefeller University, New York
PROTEIN MYRISTYLATION AS AN INTERMEDIATE STEP IN REGULATION OF MACROPHAGE FUNCTION

11:00 AM
Charles O. Rock, St. Jude's Childrens Research Hospital, Memphis
PHOSPHOINOSITOL FOR 5-BIPHOSPHATE PHOSPHOLIPASE C

11:40 AM
Shamshad Cockroft, University College London
G-PROTEINS IN PHOSPHOLIPASE C IN NEUTROPHIL ACTIVATION

PLENARY SESSION II

Saturday, October 29th

Lincoln East and Monroe

Mononuclear Phagocytes and Retroviral Disease

Monte S. Meltzer presiding

9:30 AM
Introduction: Monte S. Meltzer, Walter Reed Army Institute of Research, Washington, DC

9:40 AM
Howard E. Gendelman, Columbia University College Physicians and Surgeons, New York
HIV-MACROPHAGE INTERACTIONS

10:20 AM
Opendra Narayan, The Johns Hopkins Hospital, Baltimore
MACROPHAGES AND THE BIOLOGY OF LENTIVIRUSES

11:00 AM
Georg Stingl, University of Vienna Medical School, Vienna
CHANGES IN EPIDERMAL LANGERHANS CELLS DURING HIV INFECTION

11:40 AM
Michael S. McGrath, San Francisco General Hospital, San Francisco, CA
MACROPHAGES AS TARGET CELLS FOR HIV

PLENARY SESSION III

Sunday, October 30th

Lincoln East and Monroe

Neutrophils and Oxidase Activation

Bernard M. Babior presiding

9:30 AM
Bernard M. Babior, Research Institute of Scripps Clinic, La Jolla
PROTEIN PHOSPHORYLATION AND THE ACTIVATION OF THE RESPIRATORY BURST OXIDASE IN HUMAN NEUTROPHILS

10:00 AM
Algirdas J. Jesaitis, Research Institute of Scripps Clinic, La Jolla
CLUES TO THE FUNCTIONS OF HUMAN NEUTROPHIL CYTOCHROME b FROM ITS PURIFICATION AND STRUCTURAL CHARACTERIZATION

10:30 AM
Stewart H. Orkin, Children's Hospital, Boston
MOLECULAR GENETICS OF THE SUPEROXIDE-GENERATING SYSTEM OF NEUTROPHILS

11:00 AM
Peter E. Newburger, University of Massachusetts, Worcester
REGULATION OF THE CYTOCHROME b GENES IN PHAGOCYTES

11:30
Gary M. Bokoch, Research Institute of Scripps Clinic, La Jolla
THE ROLE OF GTP-BINDING PROTEIN IN NEUTROPHIL SIGNAL TRANSDUCTION

12:00 PM
John T. Curnutte, Research Institute of Scripps Clinic, La Jolla
STUDIES ON CHRONIC GRANULOMATOUS DISEASE USING THE CELL-FREE OXIDASE ACTIVATION SYSTEM

RETICULOENDOTHELIAL SOCIETY AWARDS

Friday, October 28th

International Ballroom East

Philip Davies presiding

2:00 PM-3:00 PM **THE RES PRESIDENTIAL AWARD COMPETITION,** papers 39-42

Ellen Buschman, Montreal General Hospital Research Institute, Montreal, Canada
ACQUIRED IMMUNITY TO M. BOVIS AND M. INTRACELLULARE IS INFLUENCED BY THE B_{cg} GENE.

Diane Hamelin-Bourassa, Montreal General Hospital Research Institute, Montreal, Canada
SUSCEPTIBILITY TO A MURINE RETROVIRUS-INDUCED IMMUNO-DEFICIENCY SYNDROME IS CONTROLLED BY THE H-2 COMPLEX.

Sarah Sporn, University of North Carolina, Chapel Hill, NC
ISOLATION OF ADHERENCE SPECIFIC cDNA CLONES FROM A MONOCYTE cDNA LIBRARY.

Linda Vespa, The Ohio State University, Columbus, OH
BIOCHEMICAL BASIS OF CONTINUOUS I-A EXPRESSION BY MACROPHAGES FROM MICE RESISTANT TO MYCOBACTERIUM BOVIS (STRAIN BCG).

3:00 PM-4:00 PM **THE RES YOUNG INVESTIGATOR AWARD COMPETITION,** papers 43-46

Ezio Bonvini, Laboratory Cell Biology/DBBP CBER/US-FDA, Bethesda, MD
THE NON-HYDROLYSABLE GUANOSINE-5'-TRIPHOSPHATE ANALOG, GUANOSINE-5'-O-(3-THIOTRIPHOSPHATE) (GTPgammaS), ACTIVATES PHOSPHOLIPASE C-MEDIATED HYDROLYSIS OF INOSITOL PHOSPHOLIPIDS IN MURINE HELPER T CELL CLONES.

Ronald G. Collman, University of Pennsylvania Medical Center, Philadelphia, PA
MONOCYTE(M)-TROPIC AND T LYMPHOCYTE(T)-TROPIC STRAINS OF HIV; REPLICATION IN CULTURED HUMAN MONOCYTES.

William J. Dreyer, Texas Children's Clinical Care, Houston, TX
NEUTROPHIL CHEMOTACTIC ACTIVATION DURING EXPERIMENTAL MYOCARDIAL ISCHEMIA AND REPERFUSION.

Karen L. MacNaul, Merck Sharp & Dohme Research Laboratories, Rahway, NJ
ANALYSIS OF IL-1 AND TNF GENE EXPRESSION IN HUMAN SYNOVIOCYTES AND MONOCYTES BY IN SITU HYBRIDIZATION.

POSTER SESSION I

Friday, October 28th, 7:30 AM-9:30AM

GRANULOCYTE ACTIVATION

Hemisphere Room

1. SELECTIVE ACTIVATION OF BOVINE NEUTROPHIL FUNCTIONS BY RECOMBINANT BOVINE INTERLEUKIN-1β. P. Canning, National Animal Disease Center, Agricultural Research Service, U.S.D.A., Ames, IA 50010.

2. GENERATION OF SUPEROXIDE ANIONS AND MYELOPEROXIDASE BY PMN IN JOINTS OF RHEUMATOID ARTHRITIS PATIENTS. B. Dularay, C.J. Elson and P.A. Dieppe, Univ. Bristol, Bristol BS8 1TD, UK.

3. ONTOGENY OF LEUKOCYTE FUNCTION: SUPEROXIDE ANION PRODUCTION BY FETAL, NEWBORN AND ADULT BOVINE NEUTROPHILS. Charles B. Clifford, D.O. Slauson, N.R. Neilsen, R.D. Zwahlen, and D.H. Schlafer, Inflammation Research Laboratory, Pathology Department, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

4. OBSERVATION OF ANTIBODY-DEPENDENT LYSIS OF RED BLOOD CELLS BY NEUTROPHILS USING NOVEL OPTICAL MICROSCOPIC TECHNIQUES. J.W. Francis, M.J. Zhou, L.A. Boxer, H.R. Petty, Biological Sciences, Wayne State Univ., Detroit, MI 48202 and Dept. of Pediatrics, Univ. of Michigan, Ann Arbor, 48109.

5. RECOMBINANT HUMAN GM-CSF IS A DIRECT STIMULATOR OF GRANULOCYTE RESPIRATORY BURST BUT REQUIRES AN ADDITIONAL STIMULUS FOR INITIATING DEGRANULATION. C. Lam, L. Klein, Sandoz Forschungsinstitut, A-1235 Vienna, Austria.

6. PAF ACTIVATION OF ACETYL-CoA: 1-ALKYL-SN-GLYCERO-3-PHOSPHOCHOLINE O^2-ACETYLTRANSFERASE, PAF SYNTHESIS AND DEGRANULATION IN RAT NEUTROPHILS. Thomas W. Doebber and Margaret S. Wu (Spon: Philip Davies), Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.

7. EFFECT OF PLATELET ACTIVATING FACTOR AND FMLP ON NEUTROPHIL CR1, CR3 AND FcR RECEPTORS AND ON BINDING AND PHAGOCYTOSIS OF OPSONIZED MICROSPHERES. J. Ogle, G. Noel, C. Ogle, M. Sramkoski, J. Alexander and G. Warden, Cincinnati Sch. Med. and Shriners Burns Institute, Cincinnati, OH 45267.

8. ENDOTOXIC INJURY TO EQUINE MICROVASCULAR ENDOTHELIUM IN VITRO IS MEDIATED THROUGH PERIPHERAL BLOOD NEUTROPHILS. Philip N. Bochsler, D.O. Slauson, M.M. Suyemoto, and N.R. Neilsen. Inflammation Research Laboratory, Pathology Department, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

9. RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (RH-GM-CSF) PRIMES AND RECRUITS RHESUS MONKEY NEUTROPHIL (PMN) RESPONSES TO N-FORMYL-L-METHIONYL-L-LEUCYL-L-PHENYLALANINE (FMLP). D.M. Linnekin, R.L. Monroy, G. Murano, R.E. Donahue and T.J. MacVittie (Spon: M.L. Patchen), Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

10. DEPRESSION OF HUMAN GRANULOCYTE CHEMILUMINESCENCE WITH SCHISTOSOMA MANSONI SCHISTOSOMULAE. I. Mofleh, A. Mahmoud, S. Al-Khwaitir, M. Alam and A. Al-Tuwaijiri, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia.

11. INHIBITION OF EOSINOPHIL SUPEROXIDE GENERATION BY MAST CELL GRANULES. K.N. Dileepan, K.M. Simpson, S. Lynch, D.J. Stechschulte (Spon: Tsuneo Suzuki), Department of Medicine, Univ. of Kansas Medical Center, Kansas City, KS 66103.

12. PROPERTIES OF EOSINOPHIL ADHESION IN VITRO. P.J. Koker, C.C. Clarke, R. Rothlein and C.D. Wegner (Spon: A.S. Rosenthal). Departments of Pharmacology and Immunology, Boehringer Ingelheim Pharma, Inc., Ridgefield, CT 06877.

13. MODULATION OF HEMATOPOIESIS IN THE GOLDEN SYRIAN HAMSTER BY THE ENDOCRINE SYSTEM. J.A. Hightower, M.J. Horacek, M.O. Dada and C.A. Blake, Sch. Med., Univ. South Carolina, Columbia, SC 29208.

14. INVOLVEMENT OF MONOCYTES IN SELECTIVE PRODUCTION OF EOSINOPHIL CHEMOTACTIC LYMPHOKINE. M. Hirashima, Kumamoto Univ. Med. Sch., Kumamoto, Japan.

HOST RESISTANCE TO TUMORS

Thoroughbred Room

15. FORMALIN-FIXED MACROPHAGES BIND TUMOR TARGETS SIMILARLY TO VIABLE MACROPHAGES. Stephen Keith Chapes, Div. of Biology, Kansas State University, Manhattan, KS 66506.

16. REGULATION OF TUMOR-INDUCED MYELOPOIESIS AND THE ASSOCIATED IMMUNE SUPPRESSOR CELLS IN MICE BEARING METASTATIC LEWIS LUNG CARCINOMAS BY PROSTAGLANDIN E_2. M.R. Young, M.E. Young and K. Kim, Research Serv., Hines V.A. Hosp., Hines, IL 60141 and Dept. Pathology, Loyola Univ. Stritch Sch. Med., Maywood, IL 60153.

17. PHENOTYPIC DIFFERENCES BETWEEN NORMAL AND TUMOR-BEARING HOST MACROPHAGES. A.D. Yurochko, R.H. Pyle, and K.D. Elgert, Dept. Biology, Microbiol. & Immunol. Section, and Veterinary Biosciences, Virginia Polytechnic Institute and State Univ., Blacksburg, VA 24061.

18. INHIBITION OF TUMOR CELL GLUTAMINE UPTAKE AS AN INDICATOR OF BOTH OXIDATIVE AND NON-OXIDATIVE CYTOTOXICITY CAUSED BY STIMULATED NEUTROPHILS. Douglas B. Learn and Edwin L. Thomas, Dept. Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101.

19. A COMPARISON OF LEUKOCYTE INFILTRATION INTO AN IMMUNOGENIC AND A NONIMMUNOGENIC MURINE TUMOR. R.A. Sneed, A.P. Stevenson, C.C. Stewart, Cell Biology Group, Los Alamos National Laboratory, Los Alamos, NM 87545.

20. THE EFFECT OF SELENIUM ON THE TUMORCYTOTOXICITY OF MOUSE PERITONEAL MACROPHAGES AND SPLEEN LYMPHOCYTES. L. Kiremidjian-Schumacher, M. Roy, H.I. Wishe, M.W. Cohen, G. Stotzky, New York Univ., College of Dentistry and Graduate School of Arts and Science, New York, NY 10010.

21. DEPRESSED CELL MEDIATED IMMUNITY IN PATIENTS WITH SEVERE INHERITED FORMS OF EPIDERMOLYSIS BULLOSA. V. Chopra[1], S. Tyring[1], S. Vaidya[2], L. Johnson[3], J.D. Fine[3], (Spon: K. Mehta), Univ. of Texas Med. Branch, Departments of Microbiology[1] and Pathology[2], Galveston, TX 77550, and Department Dermatology[3], University of Alabama at Birmingham, AL 35294.

22. THE SIGNIFICANCE OF FREE RADICAL AND FREE RADICAL SCAVENGERS IN L1210 LEUKEMIA. A. Brown and J. Lutton, New York Medical College, Valhalla, NY 10595.

23. MODULATION OF MACROPHAGE-TUMOR CELL CYTOTOXIC INTERACTIONS BY HYPERTHERMIA. J. Klostergaard, M. Barta and S.P. Tomasovic, M.D. Anderson Cancer Center, Houston, TX 77030.

HOST RESISTANCE TO INFECTION

Thoroughbred Room

24. ROLE OF ANTIBODY IN COMPLEMENT-MEDIATED KILLING OF TRITRICHOMONAS FOETUS. M.K. Aydintug, P.R. Widders (Spon: S.M. Taylor). Washington State University, College of Veterinary Medicine, Pullman, WA 99164-7040.

25. CORRELATION OF VIRULENCE IN VIVO, SUSCEPTIBILITY TO KILLING BY MURINE POLYMORPHONUCLEAR NEUTROPHILS (PMN) IN VITRO, AND PMN SUPEROXIDE ANION (O_2^-) INDUCTION IN BLASTOMYCES DERMATITIDIS (BD) ISOLATES. C.J. Morrison* and D.A. Stevens. Inst. Med. Res., Santa Clara Valley Med. Ctr., San Jose, CA 95128 and Stanford U., Stanford, CA 95304.

26. EFFECT OF IONIZING RADIATION ON THE ABILITY OF MURINE PERITONEAL CELLS TO PHAGOCYTIZE KLEBSIELLA PNEUMONIAE. D.G. McChesney, G.S. Madonna and G.D. Ledney (Spon: M. Patchen). Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

27. MITIGATION OF THE LETHAL EFFECTS OF IONIZING RADIATION BY 6,6' TREHALOSE DIESTERS. G.S. Madonna, M.L. Patchen, and G.D. Ledney. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

28. PHAGOCITOSIS OF MYCOBACTERIA BY CULTURED-HUMAN MACROPHAGES. M. Arango, G. Merizalde, L.F. Barrera, L.F. Garcia. Univ. of Antioquia, Medellin, Colombia.

29. THE RESTORATIVE EFFECTS OF GAMMA INTERFERON AND CLOFAZAMINE ON PHAGOCYTE DYSFUNCTION CAUSED BY A 25 KILODALTON FRACTION FROM MYCOBACTERIUM TUBERCULOSIS. A.A. Wadee, A.R. Rabson and R. Anderson (Spon: J. Metz). Dept. Immunology, School of Pathology of the South African Institute for Medical Research and the University of the Witwatersrand, Johannesburg, 2000, Republic of South Africa.

30. BOVINE POLYMORPHONUCLEAR NEUTROPHILIC GRANULOCYTE-PRODUCT WITH ANTIVIRAL (INTERFERON-LIKE) ACTIVITY: CHARACTERIZATION OF THE INDUCTION, SECRETION AND ACTIVITY OF "POLYFERON". H. Bielefeldt Ohmann, M. Campos, D. Fitzpatrick, M.J.P. Lawman and L.A. Babiuk. Veterinary Infectious Disease Organization, 124 Veterinary Road, Saskatoon, Saskatchewan S7N OWO, Canada.

31. INTERLEUKIN-2 INCREASES MACROPHAGE ACTIVATION BY INTERFERON-γ. A. Misefari, P. Vitale*, E. Jirillo*, S. Antonaci*, and V. Covelli*. Chairs of Immunology, University of Messina Med. School, Messina and Immunology, Clinical Medicine and Clinical Neuroanatomy, University of Bari, Med. School, Bari, Italy.

32. SEPARATE AND COMBINED EFFECTS OF rIL-1α, rTNF-α AND rFN-γ ON ANTIBACTERIAL RESISTANCE. R. Kurtz, J. Roll and C.J. Czuprynski. Univ. Wisconsin School Medicine, Madison, WI 53706.

33. A SELF-LIMITING SEMLIKI FOREST VIRUS INFECTION ACTIVATES MURINE MACROPHAGES. L.-X. Wu, K. Suryanarayana, K.-C. Lee, R.G. Marusyk and A.A. Salmi. (Spon: P.S. Morahan). Viral Pathogenesis Research Unit, Dept. of Medical Microbiol. and Infectious Diseases, and Dept. of Immunol., University of Alberta, Edmonton, Alta., Canada T6G 2H7.

34. ISOLATION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) ON RECOMBINANT HUMAN MACROPHAGE COLONY STIMULATING FACTOR (rM-CSF) TREATED HUMAN MONOCYTES: AN EFFICIENT VIRUS DETECTION SYSTEM. H. Husayni, M.S. Meltzer and H.E. Gendelman. Walter Reed Army Inst. Res., Washington, DC 20307-5100.

35. INFECTIBILITY BY THE HUMAN IMMUNODEFICIENCY VIRUS (HIV) OF HUMAN BLOOD-BORN MONOCYTES/MACROPHAGES AND CHARACTERIZATION OF INFECTED MONOCYTES/MACROPHAGES C. Meichsner, H. Rubsamen-Waigmann, R. Andreesen, M. Limbert, E. Schrinner, H Suhartono, H. von Briesen. Hoechst AG and Georg-Speyer-Haus, Frankfurt and Medizinische Universitatsklinik Freiburg, Freiburg, FRG.

36. ADMINISTRATION OF HUMAN RECOMBINANT INTERLEUKIN 2 ENHANCES ANTI-LISTERIA RESISTANCE. Mary Haak-Frendscho and Charles J. Czuprynski. School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

37. INTERLEUKIN-1-ALPHA ENHANCES PHAGOCYTOSIS AND KILLING OF M. TUBERCULOSIS AND M. AVIUM COMPLEX BY HUMAN MACROPHAGES. K. Sankaran, R. Swartz, and H. Yeager, Jr. Georgetown Univ. Medical Center, Washington, DC 20007.

38. EFFECT OF GLUCOCORTICOIDS ON MACROPHAGE INSTRINSIC RESISTANCE TO HERPES SIMPLEX VIRUS. C.W. Milligan and W.L. Dempsey. Medical College of Pennsylvania, Philadelphia, PA 19129.

MINISYMPOSIUM I

Friday, October 28, 1988

Jefferson West Room

Molecular Mechanisms Regulating Leukocyte Development

Robert Strunk presiding

4:00 PM
GENE EXPRESSION IN MACROPHAGES. Robert Strunk, M.D., Children's Hospital, Washington University Medical Center, St. Louis, MO 63110.

4:30 PM
47. SHARED 'EARLY RESPONSE' GENE EXPRESSION IN LPS-STIMULATED MACROPHAGES AND PDGF-STIMULATED FIBROBLASTS. T.A. Hamilton, C.S. Tannenbaum, and Y. Ohmori. Cleveland Clinic Foundation, Cleveland, OH 44195.

4:45 PM
48. MACROPHAGE Ia ANTIGEN EXPRESSION INDUCED BY IFNγ AND IL-4. H. Cao, R.M. Crawford, R.G. Wolff, C.A Nacy, and M.S. Meltzer. Walter Reed Army Inst. Res., Washington, DC 20307-5100.

5:00 PM
49. EXPRESSION OF c-MYC, c-FOS AND c-FMS FOLLOWING ENDOTOXIN STIMULATION OF CSF-1 INDUCED MACROPHAGE (MPH) DIFFERENTIATION. N. Ghildyal, M.J. Myers, J.K. Pullen and L.B. Schook, Lab of Molecular Immunology, Dept. of Animal Sciences, Univ. of Illinois, Urbana, IL 61820.

5:15 PM
50. PROTOONCOGENE EXPRESSION IN TUMOR-ASSOCIATED MACROPHAGES (TAM): A PARACRINE CIRCUIT IN THE REGULATION OF THE PROLIFERATION OF TAM IN MURINE SARCOMAS. A. Mantovani, E. Erba, F. Fazioli, A. Rambaldi, B. Bottazzi. Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milan, Italy.

5:30 PM
51. TUMOR-INDUCED IMMUNOSUPPRESSION: INHIBITION OF INTERLEUKIN 2 PRODUCTION BY TUMOR CELL PRODUCTS AND A p15E-RELATED PEPTIDE. David S. Nelson, Peggy Nelson, George J. Cianciolo and Ralph Snyderman. Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards NSW 2065, Australia, and Genentech, Inc., South San Francisco, CA 94080.

5:45 PM
52. SYNERGISTIC EFFECTS OF CYTOKINES ON HL-60 DIFFERENTIATION AND FUNCTIONAL ACTIVATION. J.B. Liesch, T.J. Krause, T.M. Oberyszyn, R.S. Greco and F.M. Roberston. UMDNJ/ Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

MINISYMPOSIUM II

Friday, October 28, 1988

Jefferson East Room

MECHANISMS OF GRANULOCYTE ACTIVATION

Richard B. Johnston, Jr. presiding

4:00 PM
NEUTROPHIL PRIMING. Richard B. Johnston, Jr., M.D., University of Pennsylvania, Children's Hospital, Philadelphia, PA 19104.

4:30 PM
53. DEPRESSION OF POLYMORPHONUCLEAR LEUKOCYTE (PMNL) FUNCTION INDUCED BY INFLUENZA VIRUS HEMAGGLUTININ (HA) AND SIALIC ACID (SA)-BINDING LECTINS. J.S. Abramson, L.F. Cassidy, L.S. Winkler and D.S. Lyles (Spon: D.A. Bass). Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

4:45 PM
54. INFLUENZA A VIRUS (IAV) ALTERS ACTIN DISTRIBUTION IN POLYMORPHONUCLEAR LEUKOCYTES (PMNL). J.G. Wheeler and J.S. Abramson (Spon: D.A. Bass). Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

5:00 PM
55. DIFFERENTIAL EFFECT OF PENTOXIFYLLINE ON RESPONSE OF NEUTROPHILS TO CHEMOTACTIC PEPTIDE (fMLP) AND PHORBOL ESTER (PMA). M.S. Currie, K.M.K. Rao, J. Crawford, and H.J. Cohen (Spon: J. Brice Weinberg). Duke and DVAMC, Durham, NC 27705.

5:15 PM
56. INVOLVEMENT OF PROTEIN KINASE C (PKC) IN THE ACTIVATION OF fMET-LEU-PHE (fMLF)-MEDIATED RESPIRATORY BURST IN HUMAN NEUTROPHILS (PMNs). J. Nath and A. Powledge (Spon: C. Nacy). WRAIR, Washington, DC 20307-5100.

5:30 PM
57. DEGRANULATION AND ACTIVATION OF THE RESPIRATORY BURST IN HUMAN NEUTROPHILS. D.L. Schneider, F.S. Manara and J. Chin. Dartmouth Medical School, Hanover, NH 03756.

5:45 PM
58. EFFECTS OF RECOMBINANT BOVINE INTERFERON-ALPHA AND INTERFERON-GAMMA ON BOVINE NEUTROPHIL FUNCTIONS. Allen K. Sample and Charles J. Czuprynski. Univ. of Wisconsin-Madison, Wisconsin, WI 53706.

MINISYMPOSIUM III

Friday, October 28, 1988

Lincoln West Room

INFLAMMATORY MEDIATORS

John L. Ryan presiding

4:00 PM
GANGLIOSIDES AS MACROPHAGE RECEPTORS. John L. Ryan, M.D., Ph.D., Department of Internal Medicine, Yale University School Medicine, VA Medical Center, West Haven, CT 06516.

4:30 PM
59. DIFFERENTIAL EFFECTS OF ENDOTOXINS ON THE TERMINAL DIFFERENTIATION AND FUNCTIONAL ACTIVITY OF HUMAN MONOCYTE/MACROPHAGES. R. Andreesen, W. Brugger, D. Waltersbacher, H. Sawert, L. Kanz, A. Rehm, C. Galanos, R. Engelhardt, G.W. Lohr. Medizinische Klinik, Hugstetter Strasse 55, D-7800 Freiburg, FRG.

4:45 PM
60. PRODUCTION OF TWO NOVEL NEUTROPHIL CHEMOTACTIC PEPTIDES BY LPS-STIMULATED ENDOTHELIAL CELLS. J.-M. Schroder and E. Christophers. Dept. of Dermatology, Univ. Kiel, 2300 Kiel, FRG.

5:00 PM
61. ROLE OF 1,25-DIHYDROXYCHOLECALCIFEROL AS AN AUTOCRINE MACROPHAGE REGULATOR. D.R. Katz, A. Brennan, I. Ziegler, D.S. Latchman, M. Hewison, J.L.H. O'Riordan. Univ. Coll./Middlesex Sch. Med, London W1P 8AA, U.K.

5:15 PM
62. 13-CIS RETINOIC ACID (13cRA) INCREASES MACROPHAGE PRODUCTION IN INTERLEUKIN-3 (IL-3) STIMULATED MOUSE BONE MARROW CULTURES. J.G. Bender, C.C. Stewart, and R.A. Habbersett, Dept. of Pathology, Univ. of New Mexico, Albuquerue, NM, and LANL, Los Alamos, NM.

5:30 PM
63. MODULATION OF TUMOR NECROSIS FACTOR (TNF) RELEASE BY RETINOIDS IN MURINE PERITONEAL MACROPHAGES. Kapil Mehta, Department of Clinical Immunol. and Biological Therapy, UT M.D. Anderson Cancer Center, Houston, TX 77030.

5:45 PM
64. INTERLEUKIN-4 (IL-4) EFFECTS ON MONOCYTE PGE_2 AND TUMOR NECROSIS FACTOR (TNF). C. Miller, G. Szabo and T. Takayama. Univ. Massachusetts, Worcester, MA 01655.

MINISYMPOSIUM IV

Friday, October 28, 1988

Lincoln East Room

PHOSPHOLIPIDS AND STIMULUS TRANSDUCTION PROCESSES

Filippo Rossi presiding

4:00 PM
PHOSPHOLIPID TURNOVER IN NEUTROPHIL ACTIVATION. Prof. Filippo Rossi. Istituto di Patologia Generale Universita degli Studi di Verona, 37134 Verona, Italy

4:30 PM
65. ALTERATIONS IN GTP-BINDING PROTEIN IN HUMAN NEUTROPHILS BY INFLUENZA VIRUS. Elaine L. Mills, Garry M. Bokoch, Jon S. Abramson. McGill Univ., Montreal, PQ, H3H 1P3, Res. Inst. Scripps Clinic, La Jolla, CA 92037, Bowman Gray Sch Med, Winston-Salem, NC 27103.

4:45 PM
66. LIPOPOLYSACCHARIDE-INDUCED EXPRESSION OF THE COMPETENCE GENE, KC, IN VASCULAR ENDOTHELIAL CELLS IS MEDIATED THROUGH PROTEIN KINASE C. X. Shen, T.A. Hamilton, and P.E. DiCorleto. Cleveland Clinic Research Inst., Cleveland, OH 44195.

5:00 PM
67. ROLE OF PKC IN THE CELL SURFACE EXPRESSION AND PHOSPHORYLATION OF DIFFERENTIATION ANTIGENS OF RESTING AND ACTIVATED HUMAN T. CELLS. A. Carrera, L. Cardenas, A. Tugores, M. Cebrian, F. Sanchez-Madrid, M. Lopez-Botet and M.O. de Landazuri. Servicio de Inmunologia, Univ. Autonoma. Hospital de la Princesa. c/Diego de Leon, 62- 28006 Madrid, Spain.

5:15 PM
68. PROTEIN KINASE C ISOTYPE DISTRIBUTION AND SELECTIVE ISOTYPE TRANSLOCATION WITH Ca^{2+} IN HUMAN NEUTROPHILS AND CYTOPLASTS. T. Fujiki, M.W. Rossi, W.A. Phillips, R.B. Johnston, Jr. and H.M. Korchak (Spon: L. Kilpatrick-Smith). Univ. of Pennsylvania, Philadelphia, PA 19104.

5:30 PM
69. ROLE OF PROTEIN KINASE C IN THE ACTIVATION OF LIVER MACROPHAGES. D.L. Laskin, C.R. Gardner, A.M. Pilaro and J.D. Laskin. Rutgers Univ. and UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

5:45 PM
70. THE ENHANCEMENT OF RECEPTOR-MEDIATED PHAGOCYTOSIS BY AMPHOTERICIN B MONOMETHYL ESTER (AME). S. Racis, O.J. Plescia, J.C. Mulloy, and C.P. Schaffner. Waksman Institute of Microbiology @ Rutgers-The State University, New Brunswick, NJ 08855-0759.

POSTER SESSION II

Saturday, October 29th, 7:30 AM-9:30 AM

CELLULAR DIFFERENTIATION AND MATURATION

Thoroughbred Room

71. DIFFERENTIATION OF MACROPHAGES (MP) AND NEUTROPHILS (PMN) IS ASSOCIATED WITH CHANGES IN CELLULAR PROTEIN PHOSPHORYLATION. A.A. Sirak, F.H. Mermelstein, J.D. Laskin and D.L. Laskin. Rutgers Univ. and UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

72. THE IN VITRO PROLIFERATION OF PULMONARY ALVEOLAR MACROPHAGES FROM MICE UNDER MONOCYTOPENIA OR MONOCYTOSIS. Y. Oghiso. Div. Comparative Radiotoxicology, National Institute of Radiological Sciences, Chiba 260, Japan.

73. RESIDENT PERITONEAL MACROPHAGES (MØ) ARE MAINTAINED BY LOCAL DIVISION. M.J. Melnicoff, T.C. Schmitt, P.K. Horan, and P.S. Morahan. Medical College of Pennsylvania, Philadelphia, PA 19129; and Smith, Kline and French Laboratories, King of Prussia, PA 19406.

74. IDENTIFICATION OF PULMONARY MACROPHAGE POPULATIONS IN THE MOUSE. R. Crowell, B. Lehnert, C. Mold (Spon: J. Bender). Univ. NM, Albq., NM, Los Alamos Nat. Lab., Los Alamos, NM.

75. CHARACTERISTICS OF HUMAN CD4 MONOCYTE (MØ) SUBSETS. G. Szabo, C. Miller, J. Wu and K. Kodys. Univ. Massachusetts Medical Center, Worcester, MA 01655.

76. HUMAN MONOCYTE HETEROGENEITY DEFINED BY HLA-DR EXPRESSION DOES NOT CORRELATE WITH OXIDATIVE BURST CAPABILITY. G.T. Spear, L.C. Rothberg and A.L. Landay. Rush University, Chicago, IL 60612.

77. BIOACTIVITY OF THE INSULIN RECEPTORS (IR) ON SPLENIC MACROPHAGES (Mø) IN MICE. A.P. Bautista, D.J. Fletcher and A. Volkman. School of Medicine, East Carolina University, Greenville, NC 27858.

78. MORPHOLOGICAL CHANGE OF B CELL AND MONOCYTE IN VITRO WITH PMA (4ß-PHORBOL 12-MYRISTATE 13-ACETATE). M. Matsuda, M. Ishikawa, A. Masunaga, M. Narabayashi, H. Hashimoto and Y. Imai. Yamagata Univ. Sch. Med., Yamagata, 990-23, Japan.

79. EFFECTS OF BONE MARROW SUPPRESSION WITH ^{45}Ca ON MONOCYTES AND MACROPHAGES (MØ). A. Volkman and Y. Shibata. East Carolina University School of Medicine, Greenville, NC 27858-4354.

80. INDUCTION AND CHARACTERIZATION OF HUMAN MONOCYTE-MACROPHAGE-DERIVED MULTI-NUCLEATED GIANT CELLS IN IN VITRO CULTURE. N. Hassan and S. Douglas. Division of Allergy-Immunology-BMT, Children's Hospital of Philadelphia, Univ. of Pennsylvania Medical School, Philadelphia, PA 19104.

81. DIFFERENTIAL PRODUCTION OF TUMOR NECROSIS FACTOR (TNF), MACROPHAGE COLONY STIMULATING FACTOR (CSF-1) AND INTERLEUKIN 1 (IL-1) BY HUMAN ALVEOLAR MACROPHAGES. Susanne Becker, Robert Devlin and Stephen Haskill. Environmental Monitoring and Services, Inc., U.S. Environmental Protection Agency, Chapel Hill, NC 27516.

82. INDUCTION OF DIFFERENTIATION IN HUMAN U-937 HISTIOCYTIC LEUKEMIC CELLS BY DIBUTYRYL CYCLIC ADENOSINE-3',5'-MONOPHOSPHATE (dBcAMP). A.J. Beavis, J.D. Laskin, A.A. Sirak, S.M. O'Connell and D.L. Laskin. Rutgers University and UMDNJ-RWJ Medical School, Piscataway, NJ 08854.

83. INDUCTION OF MACROPHAGE DIFFERENTIATION OF THE HUMAN PROMYELOCYTIC CELL LINE HL-60 AS DETERMINED BY FUNCTION AND IMMUNOCYTOCHEMISTRY. R.H.J. Beelen, I.L.Eestermans*, H.J. Bos*, and G.J. Ossenkoppele. Departments of Haematology and Cell Biology*, Free University Hospital and Medical Faculty*, Amsterdam, The Netherlands.

84. SURVIVAL ENHANCEMENT AND HEMOPOIETIC REGENERATION FOLLOWING RADIATION EXPOSURE: THERAPEUTIC APPROACH USING GLUCAN, A MACROPHAGE-ACTIVATOR, IN COMBINATION WITH GRANULOCYTE-COLONY STIMULATING FACTOR. M.L. Patchen, T.J. MacVittie, B.D. Solberg, L.M. Souza. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145 and AMGen, Thousand Oaks, CA.

85. PURIFICATION OF HEMATOPOIETIC PROGENITOR CELLS FROM HUMAN PERIPHERAL BLOOD. P. Law, D. Dooley, P. Alsop and L. Haiber (Spon: M. Patchen). American Red Cross, Rockville, MD 20855.

86. EFFECTS OF INTERLEUKIN-1 (IL-1) ON GRANULOCYTE AND MACROPHAGE PROGENITOR CELLS IN NORMAL AND IRRADIATED MICE. G.N. Schwartz, M.L. Patchen, and T.J. MacVittie. Armed Forces Rad. Res. Inst., Bethesda, MD 20814 and American Red Cross, Rockville, MD 20855.

87. QUANTITATIVE MODEL OF MACROPHAGE LINEAGE PROLIFERATION IN MICE. J.P. Novak, E. Skamene* and F. Gervais.* Institut de recherche d'Hydro-Quebec, Varennes, Quebec, Canada JOL 2PO and *Montreal General Hospital Research Institute, Montreal, Quebec, Canada H3G 1A4.

88. IDENTIFICATION OF THE REGULATORY SIGNALS CONTROLLING THE PROLIFERATION AND DIFFERENTIATION OF MOUSE HEMATOPOIETIC STEM CELLS. R.L. Brown*, J. Keller*. Quality Biological, Inc., Gaithersburg, MD; Biological Carcinogenesis Development Program, Program Resources, Inc., Frederick Cancer Research Facility, Frederick, MD.

89. IMMUNOHISTOCHEMICAL STUDY OF FcεR IN LYMPH FOLLICLE AND FOLLICULAR LYMPHOMA. A. Masuda*, T. Kasajima* and M. Kojima**. *Tokyo Women's Medical College, Kawadacho, Shinjuku-ku, Tokyo, Japan, **Mito Saiseikai Hospital, Futabadai, Mito, Japan.

MACROPHAGE AS EFFECTOR CELLS

Thoroughbred Room

90. ENHANCEMENT OF HUMAN MONOCYTE CYTOTOXICITY BY MULTIPLE SPECIES OF INTERFERON-ALPHA. D. Webb, K. Zoon, D. Zur Nedden, and T. Gerrard (Spon: J. Roth). FDA, Bethesda, MD 20892.

91. INTERLEUKIN-4 INDUCES TUMOR CYTOTOXICITY IN THE ABSENCE OF DETECTABLE TUMOR NECROSIS FACTOR MESSENGER RNA. R.H.G. Wolff, L.S.D. Anthony, R.M. Crawford, C.A. Nacy and M.S. Meltzer. Walter Reed Army Inst. Res., Washington, DC 20307-5100

92. MACROPHAGE RESISTANCE TO INFECTION WITH LEISHMANIA MAJOR: INDUCTION BY TUMOR NECROSIS FACTORα. Miodrag Belosevic and Carol A. Nacy. Univ. of Alberta, Edmonton, Canada and Walter Reed Army Inst. of Res., Washington, DC 20307-5100.

93. PERITONEAL CELLS OF CAPD PATIENTS, AND ESPECIALLY THE NON ADHERENT SUBPOPULATION, ARE GOOD STIMULATORS OF A MHC CLASS-II ANTIGEN DEPENDENT ALLOGENEIC MIXED LEUCOCYTE REACTION. H.J. Bos, E. de Lang, J.C. de Veld and R.H.J. Beelen*. Departments of Cell Biology and Haematology*, Medical Faculty and University Hospital*, Free University, Amsterdam, The Netherlands.

94. COMPARATIVE TUMORICIDAL ACTIVITY AND CYTOKINE SECRETION OF MACROPHAGES OBTAINED FROM DIFFERENT ANATOMICAL SITES. Viveca Sulich, Alicia V. Palleroni, Rosemary Wright, and Michael J. Brunda. Department of Oncology and Virology, Roche Research Center, Hoffmann-La Roche, Inc., Nutley, NJ 07110.

95. INVESTIGATION OF THE POSSIBLE ROLE OF MACROPHAGE TISSUE TRANSGLUTAMINASE IN FC-RECEPTOR-MEDIATED FUNCTIONS. J.A. Rummage, J. Wiggins, R.W. Leu and P.A. Johnston. The S.R. Noble Foundation, Ardmore, OK 73402.

96. GAMMA INTERFERON ENHANCED CYTOTOXICITY BY RAT LIVER MACROPHAGES IS ASSOCIATED WITH DEPRESSED PHAGOCYTOSIS. C.R. Gardner, T.W. McCloskey, and D.L. Laskin. Rutgers University, Piscataway, NJ 08854.

97. INHIBITION OF BOTH ANTIBODY-DEPENDENT AND ANTIBODY-INDEPENDENT CELLULAR CYTOTOXICITY OF MOUSE MACROPHAGES BY INHIBITORS OF C1Q SECRETION. R. Leu and M. Herriott. S.R. Noble Foundation, Biomedical Div., Ardmore, OK 73402.

CELLULAR COLLABORATION

Hemisphere Room

98. CYTOKINE INDUCED IMMUNE ACTIVATION OF HUMAN EPIDERMAL KERATINOCYTES. T.M. Oberyszyn, R.S. Greco and F.M. Robertson. UMDNJ/Robert Woood Johnson Medical School, New Brunswick, NJ 08903.

99. CYTOKINE MODULATION OF EPIDERMAL THYMOCYTE ACTIVATING FACTOR (ETAF)/INTERLEUKIN-1 (IL-1) PRODUCTION BY HUMAN EPIDERMAL KERATINOCYTES. F.M. Robertson, T.M. Oberyszyn and R.S. Greco. UMDNJ/Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

100. HEGEMONIES OF THE RETICULOENDOTHELIAL SYSTEM: BARRIER FORMING SYSTEMS OF ACTIVATED RETICULAR CELLS. L. Weiss. University Pennsylvania School Veterinary Medicine, Philadelphia, PA 19104.

101. GENETIC REGULATION OF ANTIBODY PRODUCTION TO DIFFERENT ANTIGENS IN THE MOUSE. E. Skamene, F. Gervais and D.H. Bourassa. McGill Centre for Host Resistance, Montreal General Hospital Research Institute, Montreal, Quebec, Canada H3G 1A4.

102. PHORBOL MYRISTATE ACETATE (PMA) STIMULATED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS RELEASE CHEMOTACTIC FACTOR(S) FOR HUMAN POLYMORPHONUCLEAR LEUKOCYTES (PMNL). L.E. Odekon, M.B. Weaver, P.J. Del Vecchio, T.M. Saba and P.W. Gudewicz. Albany Medical College, Albany, NY 12208.

103. PHORBOL INDUCED ADHESION OF HUMAN LYMPHOCYTES TO VASCULAR ENDOTHELIAL CELLS. L.L. Delehanty and G.M. Hebdon. Department of Chemotherapy, Glaxo Research Laboratories, Five Moore Drive, Research Triangle Park, NC 27709.

104. ADHERENCE INDUCTION OF MONOCYTE MEDIATOR GENES IS REGULATED BY EXTRACELLULAR MATRICES. D. Eierman, C. Johnson and S. Haskill. Depts. of Microbiology and Immunology, Ob/Gyn, and Lineberger Cancer Research Center, Univ. of North Carolina, Chapel Hill, NC 27599.

105. THE ROLE OF MAC-1 IN ADHESION INDUCED MEMBRANE INTERLEUKIN-1 (mIL-1) EXPRESSION. M. Labadia, R.B. Faanes, and R. Rothlein (Spon: D.C. Anderson). Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877 and Baylor College of Medicine, Houston, TX 77054.

106. FLOW CYTOMETRY CHARACTERIZATION OF MURINE MICROGLIAL CELLS MAINTAINED IN IN VITRO CULTURE. N. Hassan, J. Rothmann, S. Rifat and S. Douglas. Div. Allergy-Immunology-BMT, Children's Hospital of Philadelphia, University of Pennsylvania Medical School, Philadelphia, PA 19104.

CYTOKINES

Hemisphere Room

107. ABILITY OF INTERLEUKIN-1 TO MINIMIZE CYCLOPHOSPHAMIDE INDUCED HEMATOPOIETIC TOXICITY: EVIDENCE FOR AN EFFECT MEDIATED BY STROMAL CELLS. V.S. Gallicchio, M.J. Messino, B.C. Huelette, T.A. Kar-Mirza, D. Friedman, and M.A. Doukas. Hematology/Oncology Division, University of Kentucky Medical Center, Lexington, KY 40536.

108. SYNTHESIS OF INTERLEUKIN-1 (IL-1) BY HUMAN MONOCYTES CULTURED IN VITRO WITH AMPHOTERICIN B (AmB). D.L. Hoover, J.B. McClain, A.S. Dobek, T.A. Olson, C.A. Nacy, and B. Joshi. Walter Reed Army Medical Center and Waltr Reed Army Institute of Research, Washington, DC 20307.

109. INTERLEUKIN 1: A GROWTH FACTOR AND INDUCER OF DIFFERENTIATION FOR K-562 CELLS. A.T. Ichiki, W.D. Edmondson, J.T. Crossno, Jr., D.A. Gerard, D.A. Sugantharaj, E.G. Bamberger, C.B. Lozzio. Univ. Tennessee Med. Center/Knoxville, Knoxville, TN 37920.

110. COMPARISON OF IN VIVO EFFECTS OF HUMAN RECOMBINANT IL 1 AND IL 6 IN RADIO-PROTECTION AND INDUCTION OF EARLY AND LATE ACUTE PHASE REACTANTS. R. Neta, S.N. Vogel, G.G. Wong, and R. P. Nordan. AFRRI, USUHS, NIH, Bethesda, MD, and GI, Boston, MA.

111. HUMAN TONSILLAR LYMPHOCYTES RELEASE LYMPHOKINES THAT ALTER HUMAN IN VITRO LYMPHOCYTE MIGRATION. R.G. McFadden, K. Vickers, L.J. Fraher (Spon: P.Lala). Lawson Research Institute and University of Western Ontario, London, Canada N6A 4V2.

112. EFFECT OF CYTOKINES ON POLYMORPHONUCLEAR NEUTROPHIL (PMN) INFILTRATION IN THE MOUSE: INDUCTION OF INFILTRATION BY INTERLEUKIN 1 AND TUMOR NECROSIS FACTOR. T.A. Wiltrout,[1] A. Pilaro,[2] and T. Sayers[1] (Spon: R. Wiltrout). [1]BCDP, Program Resources, Inc. and [2]Laboratory of Experimental Immunology, BRMP, NCI-FCRF, Frederick, MD 21701-1013.

113. REQUIREMENT OF LIPID A-ASSOCIATED PROTEIN (LAP) BY rIFN-γ-PRIMED C3H/HeJ (Lps^d) MACROPHAGES (MØ)FOR TNF PRODUCTION. M. Michele Hogan and Stefanie N. Vogel. U.S.U.H.S., Bethesda, MD 20814.

114. TUMOR NECROSIS FACTOR (TNF) AND INTERLEUKIN-1 (IL-1β) mRNA HALF-LIVES ARE REGULATED BY A SHORT-LIVED RNase. J. Economou, R. Essner, K. Rhoades, W. McBride, D.L. Morton. Division of Surgical Oncology, Department of Radiation Oncology, UCLA Medical Center, Los Angeles, CA 90024.

115. AGE AND SENESCENCE: ROLE OF CACHECTIN/TUMOR NECROSIS FACTOR (TNF). S.F. Bradley, S.L. Kunkel, and C.A. Kauffman. VAMC and Univ. of Michigan, Ann Arbor, MI 48105.

116. MODULATION OF ARACHIDONIC ACID METABOLISM BY BOVINE ALVEOLAR MACROPHAGES EXPOSED TO INTERFERONS. M.G. O'Sullivan, N.J. MacLachlan, L.N. Fleischer, N.C. Olson, and T.T. Brown, Jr.. College of Veteriary Medicine, North Carolina State University, Raleigh, NC 27606.

117. REDUCTION OF COLLAGEN BIOSYNTHESIS OF VASCULAR ENDOTHELIAL CELLS BY MONOKINES IN VITRO. B. Voss, J. Rauterberg*, K.-M. Muller. Silikose-Forschungsinstitut und Institut fur Pathologie, Universitat Bochum, FRG and Institut fur Arteriosklerose-forschung, Universitat Munster, FRG*.

118. CHARACTERIZATION OF A MONOCLONAL ANTIBODY AGAINST A RECEPTOR PROTEIN FOR MOUSE GAMMA INTERFERON. M. Basu, J.L. Pace, D.M. Pinson and S.W. Russell. University Kansas Medical Center, Kansas City, KS 66103.

119. MACROPHAGE FUCOGANGLIOSIDES EXIST AS THREE SEPARATE SPECIES. C.S. Berenson, H.C. Yohe and J.L. Ryan. VAMC and Yale Univ. School of Med., West Haven, CT 06516.

MINISYMPOSIUM V

Saturday, October 29, 1988

Lincoln West Room

Anti-Microbial and Anti-Parasitic Mechanisms

Emil Skamene presiding

4:00 PM
GENETIC CONTROL OF HOST DEFENSES. Emile Skamene, M.D., McGill University, Montreal General Hospital, Montreal, Quebec, Canada H3G 1A4.

4:30 PM
120. ADMINISTRATION OF PURIFIED MONOCLONAL ANTIBODY TO L3T4 IMPAIRS THE RESISTANCE OF MICE TO LISTERIA MONOCYTOGENES INFECTION. C. Czuprynski, J. Brown, K. Young, and J. Cooley. Univ. Wisconsin School Veterinary Medicine, Madison, WI 53706.

4:45 PM
121. THE C5-SUFFICIENT A/J CONGENIC MOUSE STRAIN: INFLAMMATORY RESPONSE AND RESISTANCE TO LISTERIA MONOCYTOGENES. F. Gervais, C. Desforges and E. Skamene. McGill Center for Host Resistance, Montreal General Hospital Research Inst., Montreal, Quebec, Canada H3G 1A4.

5:00 PM
122. INTERFERON GAMMA ENHANCES HERPES SIMPLEX TYPE 1 REPLICATION IN HUMAN MONOCYTES. Janis Lazdins, Kathie Woods-Cook, Enrica Alteri and David Gangemi*. Pharmaceutical Div., Laboratory Tumor-Virology, CIBA-GEIGY Ltd, Basel 4002, Switzerland and *Univ. of South Carolina School of Medicine, Columbia, SC 29208.

5:15 PM
123. PHORBOL-INDUCED MONONUCLEAR PHAGOCYTE DIFFERENTIATION ALTERS PERMISSIVENESS TO INFLUENZA A INFECTION. J.A. Armstrong and M. Nowakowski (Spon: T. Athanassiades). S.U.N.Y. Health Science Center at Brooklyn, Brooklyn, NY 11203.

5:30 PM
124. HIV PRODUCTION BY CULTURED MACROPHAGES CAN BE REGULATED BY INTERFERON (IFN), CYTOKINES, AND BACTERIAL LIPOPOLYSACCHARIDE (LPS). R.S. Kornbluth, P.S. Oh, and D.D. Richman (Spon: S.A. Gregory). Univ. of California San Diego and the Veterans Administration Medical Center, San Diego, CA 92161.

5:45 PM
125. INVESTIGATION OF THE BLOCK IN VIRAL mRNA AND PROTEIN SYNTHESIS IN INTRINSIC RESISTANCE OF MOUSE RESIDENT PERITONEAL MACROPHAGES (PMØ) AND KUPFFER CELLS (KC) TO HERPES SIMPLEX VIRUS TYPE 1 (HSV-1). S.T. Mama, F. Anaraki, K. Leary and P.S. Morahan. The Medical College of Pennsylvania, Philadelphia, PA 19129.

MINISYMPOSIUM VI

Saturday, October 29, 1988

Lincoln East Room

Cytokine: Effector Cell Collaboration

Robert D. Schreiber presiding

4:00 PM
THE GAMMA INTERFERON RECEPTOR. Robert D. Schreiber, Ph.D., Washington University School of Medicine, St. Louis, MO 63110.

4:30 PM
126. TRANSFORMING GROWTH FACTOR β1 INHIBITS MACROPHAGE ACTIVATION FOR TUMOR CELL KILLING. Mary Haak-Frendscho, Charles J. Czuprynski and Donna M. Paulnock. Depts. Medical Microbiology and Pathobiology Sciences, University Wisconsin, Madison, WI 63706.

4:45 PM
127. TGFβ: DIFFERENTIAL SUPPRESSIVE EFFECTS ON THE ACTIVATION OF MACROPHAGES BY LK AND IFNγ FOR INTRACELLULAR DESTRUCTION OF LEISHMANIA. Barbara J. Nelson, Peter Ralph, and Carol A. Nacy. Walter Reed Army Inst. of Research, Washington, DC 20307-5100 and Cetus Corp., Emeryville, CA 94608.

5:00 PM
128. EFFECT OF TRANSFORMING GROWTH FACTOR (TGF) TYPE BETA ON MURINE INFLAMMATORY MONONUCLEAR PHAGOCYTES: INCREASED FIBRONECTIN PRODUCTION. Gideon Strassmann, James L. Cone, and Jacqueline Herrfeldt. Otsuka Pharmaceutical Co., Ltd., 9900 Medical Center Drive, Rockville, MD 20850.

5:15 PM
129. TRANSFORMING GROWTH FACTOR BETA INDUCES LEUKOCYTE INFILTRATION AND INFLAMMATION IN THE SYNOVIAL JOINT. Janice B. Allen, Larry Ellingsworth, and Sharon M. Wahl (Spon: G. Feldman). NIDR, NIH, Bethesda, MD 20892 and Collagen Corporation, Palo Alto, CA 94303.

5:30 PM
130. GROWTH REGULATION IN LYMPHOPOIESIS AND HEMATOPOIESIS BY TRANSFORMING GROWTH FACTOR-B: REGULATION OF RECEPTOR EXPRESSION. L. Ellingsworth, D. Nakayama, and J. Dasch. Collagen Corporation, Celtrix Laboratories, 2500 Faber Place, Palo Alto, CA 94303.

5:45 PM
131. TUMOR NECROSIS FACTOR (TNF) RECEPTORS ON MACROPHAGES (Mø) ARE RAPIDLY INTERNALIZED IN REPONSE TO BACTERIAL LIPOPOLYSACCHARIDE (LPS). A. Ding, E. Sanchez and C.F. Nathan. Cornell Univ. Med. College, New York, NY 10021.

MINISYMPOSIUM VII

Saturday, October 29, 1988

Monroe West Room

Mechanisms of Cellular Cytotoxicity

Stephen W. Russell presiding

4:00 PM
MACROPHAGE TUMORICIDAL ACTIVITY. Stephen W. Russell, D.V.M., Ph.D., c/o Wilkinson Laboratory, Kansas University Medical Center, Kansas City, KS 66103.

4:30 PM
132. NOVEL PHAGOCYTIC BEHAVIOR OF HUMAN NEUTROPHILS: SCISSON OF YAC TUMOR CELLS DURING ADCC. M.J. Zhou, J.W. Francis and H.R. Petty. Dept. of Biological Sciences, Wayne State University, Detroit, MI 48202.

4:45 PM
133. ROLE OF SUPEROXIDE AND ASCORBATE IN THE CYTOTOXICITY OF STIMULATED LEUKOCYTES. Douglas B. Learn and Edwin L. Thomas. Dept. of Biochemistry, St. Jude Childen's Research Hospital, Memphis, TN 38101.

5:00 PM
134. FUNCTIONAL CHARACTERIZATION OF p120 A MACROPHAGE PROTEIN WHICH COINCIDES WITH TUMORICIDAL ACTIVATION. P. Johnston. The S.R. Noble Foundation, Ardmore, OK 73402.

5:15 PM
135. TYROSINE KINASE ACTIVATION CONFERS TARGET CELL RESISTANCE TO TNF. T.C. Suen, R.U. Rodriguez, M.-C. Hung, and J. Klostergaard. University of Texas M.D. Anderson Hospital Cancer Center, Houston, TX 77030.

5:30 PM
136. N^G-MONOMETHYL-L-ARGININE (NMMA) BLOCKS KUPFFER CELL SUPPRESSION OF HEPATOCYTE PROTEIN SYNTHESIS BUT NOT TNF OR IL 1 RELEASE IN RESPONSE TO LPS. T. Billiar, R. Curran, R. Hoffman, B. Bentz, R. Simmons. Univ. of Pittsburgh, Pittsburgh, PA 15261.

5:45 PM
137. CULTURE FLUIDS FROM HIV-INFECTED HUMAN MONOCYTES ARE NEUROTOXIC AND INHIBIT PROLIFERATION OF MITO-GEN-STIMULATED LYMPHOCYTES. R.M. Crawford, H.E. Gendelman and M.S. Meltzer. Walter Reed Army Inst. Res., Washington, DC 20307-5100.

MINISYMPOSIUM VIII

Saturday, October 29, 1988

Monroe East Room

Cellular Interactions in Inflammation

Stephen J. Haskill presiding

4:00 PM
ADHERENCE AS A GENERALIZED STIMULUS FOR MONONUCLEAR PHAGOCYTES.
Stephen J. Haskill, Ph.D., University of North Carolina, Chapel Hill, NC 27514.

4:30 PM
138. MOLECULAR MECHANISMS OF ANTIGEN INDEPENDENT DENDRITIC CELL-T CELL CLUSTERING. P.D. King and D.R. Katz. Univ. Coll. and Middlesex School Medicine, London W1P 8AA, England.

4:45 PM
139. LFA-1 AND ICAM-1 IN NEUTROPHIL ADHERENCE AND TRANSENDOTHELIAL MIGRATION. C.W. Smith, S.D. Marlin, R. Rothlein, C.J. Toman, H.K. Hawkins, D.C. Anderson. Baylor Coll. of Med., Houston, TX 77054 and Boehringer Ingelheim Pharma. Corp. Ridgefield, CT 06877.

5:00 PM
140. IMMOBILIZED MONOCLONAL ANTIBODIES SPECIFIC FOR Mol (CD11b/CD18) CAN TRIGGER THE OXIDATIVE BURST OF HUMAN NEUTROPHILS. B.J. Locey, M.D. Adams, C.E. Rogers, and R.F. Todd III. Univ. Michigan Med. Sch., Ann Arbor, MI 48109.

5:15 PM
141. MONOCYTE ADHERENCE INDUCES DIFFERENTIAL GENE EXPRESSION IN MONOCYTES, ENDOTHELIAL CELLS AND STROMAL CELLS. C. Johnson, D. Eierman, S. Haskill, C. Rinehart and C.-J. Edgell. Depts. of Microbiol. and Immunol., Ob/Gyn., Pathol. and Lineberger CRC, University of North Carolina, Chapel Hill, NC 27599.

5:30 PM
142. SURFACE CONTACT MODULATION OF INFLAMMATORY MACROPHAGE ARACHIDONIC ACID METABOLISM. P.W. Gudewicz, M.B. Weaver, D.G. Moon and P.J. Del Vecchio. Dept. of Physiology, Albany Medical College, Albany, NY 12208.

5:45 PM
143. EFFECT OF TUMOR NECROSIS FACTOR ON NEUTROPHIL AND MONOCYTE MIGRATION. E. Schell-Frederick, T. Tepass, M. Kreuel, M. Pfreundschuh, M. Schaadt and V. Diehl. Medizinische Universitaetsklinik I, D-5000 Cologne 41, FRG.

POSTER SESSION III

Sunday, October 30th, 7:30 AM-9:30 AM

MACROPHAGE ACTIVATION AND REGULATION

Thoroughbred Room

144. CHARACTERIZATION OF THE DEFECTIVE P/J MOUSE MACROPHAGE RESPONSE TO ACTIVATION SIGNALS. Anne H. Fortier, David S. Finbloom and Carol A. Nacy. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

145. TRANSGLUTAMINASE LEVELS AND IMMUNOLOGIC FUNCTIONS OF BCG-ELICITED MOUSE PERITONEAL MACROPHAGES ISOLATED BY CENTRIFUGAL ELUTRIATION. V. Kera[1] and K. Mehta[2]. University of Texas Medical Branch[1], Galveston, TX 77550 and UT M.D. Anderson Hospital[2], Houston, TX 77030.

146. DIFFERENTAIL EFFECT OF RECOMBINANT GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) ON HUMAN MONOCYTES AND ALVEOLAR MACROPHAGES. M.J. Thomassen, B.P. Barna, H. Wiedemann, M. Farmer, R. Bukowski and M. Ahmad. Cleveland Clinic, Cleveland, OH 44106.

147. DIFFERENTIAL EFFECTS OF LIPOSOME-INCORPORATION ON LIVER MACROPHAGE-ACTIVATING POTENCIES OF LPS, LIPID A AND MDP: DIFFERENCES IN SUSCEPTIBILITY TO LYSOSOMAL ENZYMES. Gerit Scherphof[1], Jan Dijkstra[2] and Toos Daemen[1]. [1]University Groningen, The Netherlands and [2]Veterans Administration Medical Center, West Haven, CT 06516.

148. INTERLEUKIN 2 AND INTERFERON GAMMA ACT SYNERGISTICALLY TO PRIME AND ACTIVATE KUPFFER CELLS. R. Curran, T. Billiar, B. Bentz, F. Ferrari, R. Simmons. Univ. of Pittsburgh, Pittsburgh, PA 15261.

149. DECREASED EXPRESSION OF IMMUNE ASSOCIATED (Ia) ANTIGEN AND PRODUCTION OF TUMOR NECROSIS FACTOR BY LIPOPOLYSACCHARIDE ACTIVATED LIVER MACROPHAGES. T.W. McCloskey, C.R. Gardner, F.M. Robertson and D.L. Laskin. Rutgers University and UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

150. cAMP SELECTIVELY SUPPRESSES LPS-INDUCED MACROPHAGE GENE EXPRESSION. C.S. Tannenbaum and T.A. Hamilton. Cleveland Clinic Foundation, Cleveland, OH 44195.

151. ACTIVATION OF MONONUCLEAR PHAGOCYTES BY BACTERIAL PEPTIDOGLYCANS: POSSIBLE ROLE IN THE PATHOGENESIS OF ARTHRITIS. M. Poy, J. Allen, H. Wong, L. Wahl and S. Wahl (Spon: G. Feldman). NIDR, NIH, Bethesda, MD 20892.

152. THE CONDENSED TANNIN FROM COTTON MILL DUST PROMOTES THE RELEASE OF ARACHIDONIC ACID FROM RABBIT ALVEOLAR MACROPHAGES. M. Rohrbach, T. Kreofsky, J. Russell (Spon: R. Ritts). Mayo Clinic, Rochester, MN 55905 and SUNY-Buffalo, Buffalo, NY 14218.

153. HUMORAL FACTORS IN THE INDUCTION OF PROSTAGLANDIN E_2-PRODUCING MACROPHAGES IN VITRO. Y. Shibata. Dept. of Pathology, East Carolina University School of Medicine, Greenville, NC 27858, and Fujisaki Cell Center, Hayashibara Biochemical Laboratories, Inc., Okayama 702, Japan.

154. ASSESSMENT OF MACROPHAGE INFILTRATION AND MATURATION IN THE GUINEA PIG LUNG IN RESPONSE TO AN ACUTE INFLAMMATORY STIMULUS. D.K. Newton-Nash and P. Abramoff. Marquette University, Department of Biology, Milwaukee, WI 53233.

155. COMBINATION OF C-REACTIVE PROTEIN (CRP) AND INTERLEUKIN-2-ENHANCES HUMAN MONOCYTE TUMORICIDAL ACTIVITY. B. Barna, M.J. Thomassen, S. Malcolm-Kohn, J. Pettay and S.D. Deodhar. Cleveland Clinic, Cleveland, OH 44195-5131.

156. REGULATION OF MACROPHAGE ACTIVATION BY A COLONY STIMULATING FACTOR. G. Frendl and D.I. Beller (Spon: C.L. Miller). Boston University Medical Center, Boston, MA 02118.

157. BRIEF EXPOSURE TO Ca++ CAUSES INCREASED EXPRESSION OF MEMBRANE COMPLEMENT RECEPTORS AND ADHESION MOLECULES BY HUMAN PERIPHERAL BLOOD MONOCYTES AND LYMPHOCYTES ISOLATED IN THE PRESENCE OF EDTA. S.D. Apfelroth and M. Nowakowski. State University of N.Y. at Brooklyn, NY 11203.

158. DOWN REGULATION OF INTERFERON(IFN)-α AND IFN-β ON MACROPHAGE ACTIVATION BY IFN-γ. P. Vitale*, A. Misefari, E. Jirillo*, V. Covelli* and S. Antonaci*. Chairs of Immunology, University of Messina Med. School, Messina and Immunology, Clinical Neuroanatomy and Clinical Medicine, University of Bari Med. School, Bari, Italy.

159. IRON-LOADING INCREASES IL-1 SECRETION IN P388D1 CELLS BY DECREASING MEMBRANE ASSOCIATED ACTIVITY. S. Shedlofsky, C. McClain, D. Cohen, J. Robinson, and K. Keaton. VA Hospital and University Kentucky, Lexington, KY 40511.

160. DOWN REGULATION OF HUMAN PERIPHERAL MONOCYTE INTERLEUKIN-1 BY INTERLEUKIN-4. R. Essner, J.S. Economou, K. Rhoades, W. McBride, and D.L. Morton. Division Surgical Oncology, Factor Bldg., UCLA Medical Center, Los Angeles, CA 90024.

161. EXPRESSION OF CHONDROITIN SULFATES IN ACTIVATED RAT LIVER MACROPHAGES. A. Dokidis, C.R. Gardner, F.M. Robertson, D.L. Laskin and J.D. Laskin. UMDNJ-RW Johnson Medical School and Rutgers University, Piscataway, NJ 08854.

162. KINETICS AND REGULATION OF BOVINE ALVEOLAR MACROPHAGE PROCOAGULANT ACTIVITY. Bruce D. Car, D.O. Slauson, M.M. Suyemoto, and N.R. Neilsen. Inflammation Research Laboratory, Pathology Department, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

163. ROLE OF EPSTEIN-BARR VIRUS GENE EXPRESSION IN HISTIOCYTIC ACTIVATION. Z. Dreyer, B. Dowell and K. McClain (Spon: D. Anderson). Dept. of Pediatrics, Baylor College of Medicine, Houston, TX 77030.

164. THREE DIMENSIONAL STRUCTURE OF BEIGE MOUSE MACROPHAGE LYSOSOMES. P. Strausbauch and N. Sehgal. East Carolina University, Greenville, NC 27858-4354.

165. SURFACE DISTRIBUTION OF Fc RECEPTORS IN RAT ALVEOLAR MACROPHAGES ACTIVATED IN VIVO BY POLY I:C. P.J. Bugelski, A.M. Klinkner, A. Kelley, P.C. Meunier and R.A. Weiss. Smith Kline & French Laboratories, Philadelphia, PA.

166. *RECEPTOR MEDIATED BINDING* OF C-REACTIVE PROTEIN (CRP) TO HUMAN MONOCYTES AND THE U937 MONOCYTE CELL LINE OCCURS VIA RECEPTORS DISTINCT FROM IgG Fc-RECEPTORS. J.M. Tebo and R.F. Mortensen. The Ohio State University, Columbus, OH 43210.

167. IMMUNOLOGIC MECHANISMS OF A TRAUMA ASSOCIATED GLYCOPEPTIDE. A.N. Ozkan, S. Tompkins, S. Gregory, D.B. Hoyt. Univ. of California Medical Center, San Diego, CA 92103 and Univ. of Chicago Medical Center, Chicago, IL 60637.

LYMPHOCYTES AND NK CELLS

Hemisphere Room

168. CHARACTERIZATION OF MUCOSAL LYMPHOCYTES OF RABBIT GUT-ASSOCIATED LYMPHOID TISSUE. A. Ruiz, M. Roy and M. Gordon (Spon: C. Nacy). Walter Reed Army Institute of Research, Washington, DC 20307 and N.I.A.I.D., Bethesda, MD 20892.

169. MIXED RBC-LEUKOCYTE AGGREGATES AND STABLE AUTOLOGOUS E-ROSETTES IN HUMAN BLOOD CULTURES STIMULATED BY TYPHUS GROUP RICKETTSIAE. T. Khavkin, M. Carl, M. Kuchler, M.J. Liao and D. Testa. Interferon Science, Inc., New Brunswick, NJ 08901 and Naval Med. Res. Inst. Bethesda, MD 20814.

170. CHANGES IN LYMPHOCYTE SUBSETS IN PATIENTS SUFFERING FROM ACUTE THERMAL INJURY. F. Chrest, C. White, Y. Guo, W. Adler, A. Munster and R. Winchurch. Johns Hopkins Univ. Sch. Med. and Gerontology Res. Ctr. NIA, Baltimore, MD 21224.

171. MODULATION OF ANTIBODY RESPONSE BY IMMOBILIZATION STRESS. W. Roscoe, L. Ross and C.E. Taylor. The Medical College of Pennsylvania, Philadelphia, PA.

172. PRECURSOR DULL Ly-1 THYMOCYTES ALSO CONTAIN NK PRECURSORS. B.J. Mathieson, T. Gregorio, J. Wine and L. Mason (Spon: R. Wiltrout). Laboratory of Experimental Immunology, BRMP, NCI-FCRF, Frederick, MD 21701-1013.

173. DIFFERENTIAL EFFECTS OF PROTEIN KINASE C ACTIVATORS ON RAT LARGE GRANULAR LYMPHOCYTE (LGL) CHEMOTAXIS AND CYTOTOXICITY. A.M. Pilaro, T.J. Sayers* and R.H. Wiltrout. Laboratory of Experimental Immunology, BRMP, and *BCDP, Program Resources, Inc., NCI-FCRF, Frederick, MD 21701-1303.

174. AUGMENTATION OF MURINE SPLENIC NATURAL KILLER (NK) CELL ACTIVITY FOLLOWING SINGLE AND MULTIPLE INJECTION REGIMENS OF SOLUBLE GLUCAN. D. Williams, E. Jones, H. Pretus, R. McNamee and W. Browder. Departments of Physiology and Surgery, Tulane University School of Medicine, New Orleans, LA 70112.

175. THE EFFECT OF ACUTE IN VIVO EXPOSURE TO 4 ppm NO_2 ON MURINE SPLENIC T LYMPHOCYTE SUBPOPULATIONS. K.S. Damji and A. Richters. University Southern California Sch. of Med., Dept. of Pathology, Los Angeles, CA 90033.

176. ENDOTOXINS WITH DIFFERENT STRUCTURES BIND TO DISTINCT B LYMPHOCYTE GANGLIOSIDES. L. Brown, S. Vukajlovitch, D.C. Morrison, and J.L. Ryan. VAMC and Yale Univ. School of Medicine, West Haven, CT 06516, and Kansas University Sch. of Medicine, Kansas City, KS 66101.

192. LYMPHOKINE - ACTIVATED KILLER (LAK) CELLS DISCRIMINATE BETWEEN SMALL ALLOGENEIC AND SYNGENEIC LYMPHOCYTES IN VITRO. B. Rolstad, J.T. Vaage and S. Fossum. Anatomical Institute, University of Oslo, Karl Johans Gate 47, N-0162 Oslo 1, Norway.

IMMUNOPATHOLOGICAL CONSEQUENCES OF HOST DEFENSE

Hemisphere Room

177. SYNOVIAL TISSUE MACROPHAGES IN HUMAN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS. Shobha R. Chitneni, Karen L. Patton and J. Brice Weinberg. V.A. and Duke University Medical Centers, Durham, NC 27705.

178. THE MECHANISM OF SPECIFIC UNRESPONSIVENESS TO RAT CARDIAC ALLOGRAFTS INDUCED BY B-LYMPHOCYTE PRETREATMENT. Soji F. Oluwole, Tarik Wasfie, Mark A. Hardy. Columbia University, New York, NY 10032.

179. TRANSFORMED FIBROBLAST, "FIBROHISTIOCYTOID CELL" INDUCTION IN VITRO. M. Takagi, M. Yamakawa, K. Tajima, S. Ohe, T. Osanai and Y. Imai. Yamagata University School Medicine, Yamagata, Japan 990-23.

180. MONOCYTE ACTIVATION IN AIDS RELATED COMPLEX (ARC). C. Spillert, F. Tecson-Tumang, J. Lombardo, and E. Lazaro. UMDNJ-New Jersey Medical School, Newark, NJ 07103-2757.

181. EVALUATION OF KUPFFER CELL Fc RECEPTOR FUNCTION IN VIVO AFTER INJURY. D.J. Loegering, F.A. Blumenstock, B.G. Cuddy. Dept. Physiology, Albany Medical College, Albany, NY 12208.

182. MORPHOLOGICAL, IMMUNOHISTOCHEMICAL AND ENZYME HISTOCHEMICAL STUDY OF INTRA-THYROIDAL LYMPHOID FOLLICLES. Y. Imai, M. Yamakawa, K. Tajima, M. Takagi, S. Ohe, and T. Osanai. Yamagata University Sch. Medicine, Yamagata, Japan, 990-23.

183. FUNCTIONAL SIGNIFICANCE OF DECREASED SERUM ZINC IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) DISEASE. J. Falutz, C.M. Tsoukas, G. Deutsch (Spon: E. Skamene). Montreal General Hospital, McGill University, Montreal, Quebec, Canada, H3G 1A4.

IMMUNOPHARMACOLOGY

Hemisphere Room

184. ANTINFLAMMATORY EFFECTS OF NON-STEROIDAL ANTIINFLAMMATORY DRUGS (NSAIDs) INDEPENDENT OF AN EFFECT ON THE CYCLOOXYGENASE ENZYME. M. Forrest, V. Zammit, and P. Brooks (Spon: G. Koo). Dept. of Rheumatology, Royal North Shore Hospital, St. Leonards, 2065, NSW, Australia.

185. IMMUNIZATION AGAINST TRYPANOSOME CRUZI: ADJUVANTICITY OF GLUCAN. D. Williams, R. Yaeger, W. Browder, R. McNamee, E. Jones and H. Pretus. Departments of Physiology, Tropical Medicine and Surgery, Tulane University School of Medicine and School of Public Health and Tropical Medicine, New Orleans, LA 70112.

186. L-659,286, A SUBSTITUTED BETA-LACTAM, IS A SELECTIVE INHIBITOR OF EXTRACELLULAR HUMAN POLYMORPHONUCLEAR LEUKOCYTE ELASTASE. R.J. Bonney, A. Maycock, P. Dellea, K. Hand, D. Osinga, D. Fletcher, R. Mumford, J. Stolk, P. Davies and J. Doherty. Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.

187. IMMUNE STIMULATION FOLLOWING SINGLE OR MULTIPLE INJECTIONS OF SOLUBLE GLUCAN: TEMPORAL RELATIONSHIP. H. Pretus, R. McNamee, E. Jones, W. Browder and D. Williams. Departments of Physiology and Surgery, Tulane University School of Medicine, New Orleans, LA 70112.

188. PHARAMACOLOGIC REGULATION OF THE DEVELOPMENT OF STREPTOCOCCAL CELL WALL INDUCED ARTHRITIS IN RATS. Jennifer Swisher, Janice Allen, Gerald Feldman, Larry Wahl and Sharon Wahl. NIH, Bethesda, MD 20892.

189. CELLULAR IMMUNITY IN MULTIPLE MYELOMA (MM): MODULATION BY RANITIDINE. H. Nielsen, H.J. Nielsen, K. Klarlund, A. Drivsholm, F. Moesgaard, H. Kehlet. Statens Seruminstitut, Hvidovre Hospital and Rigshospitalet, Copenhagen, Denmark.

190. HETEROGENEITY OF LYMPHOCYTE CYCLOSPORINE A BINDING SITES AND THEIR EFFECT ON MEMBRANE FLUIDITY. C.D. Niebylski and H.R. Petty. Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

191. ARACHIDONIC ACID METABOLISM IN CAPRINE ALVEOLAR MACROPHAGES. M.D. Englen, S.M. Taylor, W.W. Laegreid, R.M. Silflow, K.L. Banks and K.W. Leid. Dept. of Veterinary Microbiology and Pathology, Washington State Univ., Pullman, WA 99164-7040.

WORKSHOPS AND CLUBS*

Special Afternoon Workshop on Kinetic Methods in Leukocyte Biology
(By Special Registration only)

Organized by: Larry Sklar, Scripps Clinic and Research Foundation, La Jolla, CA

Thursday, October 27, 1988

1:00 - 5:00 PM **Introduction to Spectrofluorometric and Flow Cytometric Methods** — Military Room
Geneva Omann, University of Michigan, Ann Arbor, MI and
Bruce Seligmann, Ciba-Geigy Corporation, Summit, NJ

Friday, October 28, 1988

1:00 - 5:00 PM **Multiparameter Analysis and Spectrofluorometry** — Military Room
Geneva Omann and Larry Sklar

Saturday, October 29, 1988

1:00 - 5:00 PM **Multiparameter Analysis in Flow Cytometry** — Military Room
Bruce Seligmann and Larry Sklar

CLUBS*

Friday, October 28, 1988 — Dupont Room

12:30 - 2:00 PM **Aging and the Immune Response**
Organized by: Andy C. Reese, Medical College of Georgia, Augusta, GA
Carol A. Kauffman, VA Hospital, Ann Arbor, MI

Friday, October 28, 1988 — Dupont Room

7:00 - 8:30 PM **HIV Virus and Macrophages**
Organized by: Richard S. Kornbluth, VA Medical Center, San Diego, CA
Monte S. Meltzer, Walter Reed Army Institute of Research, Washington, DC

Saturday, October 29, 1988 — Dupont Room

12:30 - 2:00 PM **Neutrophil Proteases in Inflammation**
Organized by: Philip Davies, Merck Sharp and Dohme Research Labs, Rahway, NJ
Robert J. Bonney, Merck Institute for Therapeutic Research, Rahway, NJ

*Other clubs may be organized by participants at the meeting and will be listed at the Registration Desk.

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ABSTRACTS

1

SELECTIVE ACTIVATION OF BOVINE NEUTROPHIL FUNCTIONS BY RECOMBINANT BOVINE INTERLEUKIN-1β.
P. Canning. National Animal Disease Center, Agricultural Research Service, U.S.D.A., Ames, IA 50010.

The effects of recombinant bovine interleukin-1β (IL-1β) upon *in vitro* bovine neutrophil functions were determined. Peripheral blood neutrophils exposed to various concentrations of IL-1β exhibited a dose-dependent suppression of their ability to migrate under agarose. Incubation of neutrophils with IL-1β alone did not effect their ability to ingest radiolabelled *S. aureus*, produce hydrogen peroxide or release elastase. However, pretreatment of phagocytes with IL-1β resulted in a dose-dependent enhancement of opsonized zymosan-induced hydrogen peroxide production. In contrast, IL-1β had no effect upon the ability of opsonized zymosan-stimulated neutrophils to release elastase from primary granules. Evaluation of the time course required for IL-1β to exert its effects indicated that preincubation of neutrophils with IL-1β for as little as 15 minutes was sufficient to induce suppression of migration and enhancement of opsonized zymosan-induced hydrogen peroxide production. These results suggest that IL-1β functions as a weak direct activator of neutrophils and that IL-1β may prime the phagocyte resulting in an enhanced oxidative metabolic response to other stimuli.

2

GENERATION OF SUPEROXIDE ANIONS AND MYELOPEROXIDASE BY PMN IN JOINTS OF RHEUMATOID ARTHRITIS PATIENTS. B.Dularay, C.J. Elson and P.A. Dieppe Univ. Bristol, Bristol BS8 1TD, UK.

Polymorphonuclear leukocytes (PMN) from normal individuals were stimulated by some (8/35) rheumatoid arthritis (RA) synovial fluids (SF) to generate superoxide anions and by some (8/20) to degranulate as judged by release of myeloperoxidase and/or beta-glucuronidase. Some SF that stimulated degranulation did not stimulate production of superoxide anions. Removal of IgG aggregates from the SF resulted in loss of both superoxide anion generating and degranulative activity. SF that failed to stimulate the oxidative response inhibited aggregated IgG induced but not FMLP or PMA induced superoxide anion generation. By contrast, SF that failed to stimulate myeloperoxidase release inhibited the activity of myeloperoxidase but not that of beta-glucuronidase. Myeloperoxidase activity was found in 41/50 RA SF and those containing low or no myeloperoxidase activity exhibited most myeloperoxidase inhibitory activity. Thus in RA joints, PMN may be stimulated to generate superoxide anions by some IgG aggregates if the SF inhibitor levels are low and also to release myeloperoxidase which may be inactivated in the presence of a distinct inhibitor. Preliminary experiments to identify the two inhibitors have shown that both are present in normal human and RA serum at high levels and that both are non-dialysable, heat stable molecules distict from rheumatoid factor and albumin.

3

ONTOGENY OF LEUKOCYTE FUNCTION: SUPEROXIDE ANION PRODUCTION BY FETAL, NEWBORN AND ADULT BOVINE NEUTROPHILS. Charles B. Clifford, D.O. Slauson, N.R. Neilsen, R.D. Zwahlen, and D.H. Schlafer, Inflammation Research Laboratory, Pathology Department, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

Newborn calves, like human infants, are uniquely susceptible to bacterial infections. This increased susceptibility may be related to defects in newborn polymorphonuclear leukocyte (N-PMN) defensive functions. It remains unclear whether reported deficits in N-PMN function are an unique perinatal suppression phenomenon or a PMN maturational defect. We therefore compared the ability of bovine N-PMN (less than 24 hrs old), N-PMN (7 to 10 days of age), fetal PMN (F-PMN, 210-220 days gestational age) and adult PMN (A-PMN) to generate superoxide anion (O_2^-) as an indicator of respiratory burst activity. Citrated blood was collected and PMN isolated to greater than 95% purity and 98% viability. O_2^- generation was measured as the superoxide dismutase-inhibitable (50 μg/ml) reduction of ferricytochrome C (2 mg/ml) after stimulation with phorbol myristate acetate (PMA, 2 μg/ml). O_2^- production kinetics were measured (37°C, 550nm) for 5 minutes after the initial lag period and the total nmol O_2^-generated calculated using the molar extinction coefficient for ferricytochrome C. PMN from newborn and 7-10 day old calves produced significantly less O_2^- (5.7 ± 0.8 nmol $O_2^-/10^6$ cells/5 min, $p<0.01$) than did A-PMN (n=14) (9.6 ± 2.1 nmol $O_2^-/10^6$ cells/5 min) or F-PMN (n=4) (10.7 ± 0.7 nmol $O_2^-/10^6$ cells/5 min). There was no difference in measured lag time between N-PMN and A-PMN, but F-PMN had significantly reduced ($p<0.01$) mean lag time. The data indicated that bovine N-PMN have a decreased ability to generate O_2^- in response to PMA stimulation which persists for at least 7 to 10 days, and that this functional decrement may be a perinatal suppression phenomenon rather than a developmental abnormality as F-PMN produced O_2^- as well as did adult PMN. (Supported by U.S.D.A. Grant Funds)

4

OBSERVATION OF ANTIBODY-DEPENDENT LYSIS OF RED BLOOD CELLS BY NEUTROPHILS USING NOVEL OPTICAL MICROSCOPIC TECHNIQUES. J.W. Francis, M.J. Zhou, L.A.Boxer, H.R. Petty, Biological Sciences, Wayne State Univ., Detroit, MI 48202 and Dept. of Pediatrics, Univ. of Michigan, Ann Arbor, 48109.

We have developed methods to directly observe antibody-dependent destruction of red blood cells (RBCs) by polymorphonuclear leukocytes (PMN). PMNs were incubated with IgG-coated sheep or human RBCs at 37°C. Phagocytosis and lysis of RBCs were observed by video-intensified microscopy (VIM). Absorption spectrophotometry showed that exposure of hemoglobin (Hb) to superoxide anions decreases Soret band intensity and shifts it to lower wavelengths. Oxidation of Hb is viewed within RBCs using VIM in conjunction with illumination at 430nm. Using this method, extracellularly bound RBCs were observed to be sequentially oxidized by PMNs. This oxidation was confirmed by the finding that PMNs from CGD patients did not influence absorption of IgG-coated RBCs at 430nm. Oxidation of extracellular RBCs was fast (>1 sec.) compared to the intracellular oxidation of Hb. To observe events surrounding oxidation, the membrane and cytosol were labeled with fluorescein (FLRBC) or eosin Y (EORBC), respectively. Fluorescein predominantly labels band 3. FLRBC membrane remained visible after Hb oxidation of bound RBCs. In some cases sheep and hereditary spherocytic RBCs demonstrated clustering of band 3 at the effector-target interface. Swelling of ingested FLRBCs was apparent in spherocytes. Ingested FLRBCs eventually appeared as remnants inside swollen Hb-filled phagosomes. During cytolysis eosin fluorescence diffused away from bound EORBCs or filled phagosomes. The fluorescent diffusion gradient at the membrane rupture site suggests that a pore 30nm in diameter is formed. The site of pore formation is not found at the target-effector interface. These studies provide the first direct microscopic observations of RBC oxidation and lysis.

5

RECOMBINANT HUMAN GM-CSF IS A DIRECT STIMULATOR OF GRANULOCYTE RESPIRATORY BURST BUT REQUIRES AN ADDITIONAL STIMULUS FOR INITIATING DEGRANULATION. C. Lam*, L. Klein, Sandoz Forschungsinstitut, A-1235 Vienna, AUSTRIA.

The direct stimulation of human granulocyte respiratory burst and induction of degranulation by recombinant human granulocyte-macrophage colony stimulating factor (rH GM-CSF) were investigated. In contrast to earlier reports, the GM-CSF directly stimulated the granulocytes to produce O_2^- in the absence of an additional stimulus in a dose-dependent manner. The total amount of the O_2^- induced by the GM-CSF was significantly higher than that produced by control cells. For example, whereas control cells produced 2.5 ± 1.5 nanomoles $O_2^-/10^6$ cells/10 minutes, the granulocytes stimulated with an optimal amount of GM-CSF (1ng/ml) produced on the average of 8.1 ± 0.9 nanomoles of O_2^- ($p \leq 0.05$). Besides its direct effect, the GM-CSF was also a potent priming stimulus for enhanced production of O_2^- in response to f-met-leu-phe. At the optimal concentration of GM-CSF (1ng/ml), the total O_2^- production increased to 24.2 ± 0.6 nanomoles $O_2^-/10^6$ cells/10min ($p \leq 0.001$) in response to a second stimulus. Contrary to the data on respiratory burst, GM-CSF was not a direct stimulator of degranulation but primed the granulocytes in a dose-dependent fashion for the f-met-leu-phe-enhanced secretion of elastase from azurophil granules. Taken together, the results show that E.coli-derived GM-CSF can selectively activate granulocyte oxidative metabolism directly but requires an additional stimulus for initiating the degranulation of lysosomal enzymes.

6

PAF ACTIVATION OF ACETYL-CoA: 1-ALKYL-SN-GLYCERO-3-PHOSPHOCHOLINE O^2-ACETYLTRANSFERASE, PAF SYNTHESIS AND DEGRANULATION IN RAT NEUTROPHILS. Thomas W. Doebber and Margaret S. Wu (Spon: Philip Davies). Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.

We previously demonstrated that PAF stimulates the PAF-synthesizing acetyltransferase and PAF synthesis in isolated neutrophils (T.W. Doebber and M.S. Wu, Proc. Natl. Acad. Sci. USA 84: 7557, 1987). We have further characterized these neutrophil responses to PAF along with PAF induction of neutrophil degranulation (secretion of β-glucuronidase and myeloperoxidase). PAF stimulation of rat PMNs resulted in activation of the acetyltransferase and PAF synthesis (incorporation of [^{3}H]acetate into [^{3}H]PAF) that were both substantial by 30 seconds and maximal by 1 min after agonist addition. By 4 min after agonist addition, little or no [^{3}H]PAF remained probably due to the action of the neutrophil acetylhydrolase. Activation of acetyltransferase was the most sensitive response to PAF occurring at 1-10 nM PAF while PAF synthesis and degranulation steadily increased with 30 nM to 3 μM PAF. Acetyltransferase activation and degranulation also differed markedly in their sensitivity to inhibition by the very potent PAF antagonist L-659,989 [(±)-trans-2,5-bis(3,4,5-trimethoxyphenyl) tetrahydrofuran]. At 3 x 10^{-7} M PAF, the induction of acetyltransferase experienced only 13% inhibition by 10^{-7} M L-659,989 while degranulation was 100% inhibited. However, Schild plot analyses of L-659,989 dose-response inhibition of acetyltransferase activation and degranulation induced by different levels of PAF resulted in very similar slopes and almost identical K_B values for L-659,989 of 3 x 10^{-9} M. PAF induction of acetyltransferase exhibited no dependence on cytochalasin B and was maximal at room temperature while degranulation required cytochalasin B (5 μg/ml) and was maximal only at 37°C. Both neutrophil responses required extracellular Ca^{+2}. The data is consistent with acetyltransferase activation and degranulation involving just one PAF receptor type on the neutrophil but requiring different levels of PAF receptor occupancy.

7

EFFECT OF PLATELET ACTIVATING FACTOR AND FMLP ON NEUTROPHIL CR1, CR3 AND FcR RECEPTORS AND ON BINDING AND PHAGOCYTOSIS OF OPSONIZED MICROSPHERES. J.Ogle, G.Noel, C.Ogle, M.Sramkoski, J.Alexander and G. Warden. Cincinnati Sch. Med. and Shriners Burns Institute, Cincinnati, OH 45267.

Platelet activating factor, PAF, has various direct physiological effects on neutrophils and also on indirect priming action in which the cells acquire an enhanced response to a stimulus such as the chemotactic peptide FMLP. We were interested in the effect of incubation of neutrophils with PAF alone and followed by incubation with FMLP on CR1, CR3 and FcR levels and on the binding and phagocytosis of opsonized polystyrene microspheres. In seven experiments, PAF alone caused a 1 to 2-fold increase in CR1, 2 to 4-fold increase in CR3 and a 50 to 80% increase in FcR; had little effect on binding of C3b-coated microspheres and caused a 35% to 110% increase in phagocytosis of C3b-IgG-coated microspheres. However, when neutophils were pretreated with PAF (10^{-10}-10^{-5}M) there was a dose dependent decrease up to 50% in phagocytosis of the microspheres. The rate of increase in CR1 with increasing PAF concentration was slow and approached a maximum at 10^{-5}M PAF whereas phagocytosis was maximum at 10^{-7}M and remained constant with further increasing PAF concentration.

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ENDOTOXIC INJURY TO EQUINE MICROVASCULAR ENDOTHELIUM *IN VITRO* IS MEDIATED THROUGH PERIPHERAL BLOOD NEUTROPHILS Philip N. Bochsler, D.O. Slauson, M.M. Suyemoto, and N.R. Neilsen. Inflammation Research Laboratory, Pathology Department, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

Morphologic damage to the vascular endothelium has been observed *in vivo* in some models of endotoxemia, but in most cases it is uncertain whether this is a direct effect of endotoxin (LPS) or is mediated through other means. We examined the potential direct cytotoxic effects of LPS to cultured endothelium using equine microvascular endothelium (EMVE) as a model because of the marked sensitivity of the equine species to LPS. We also investigated the role of LPS-exposed peripheral blood neutrophils (PMN) in mediating injury to EMVE. Confluent monolayers of ^{51}Cr-labeled EMVE were exposed to 1.0 to 500 μg/ml *E. coli* 0111:B4 or *S. abortus equi* LPS for up to 24 hours. No evidence of cytotoxicity as assessed by phase contrast microscopy and ^{51}Cr release was detected for the EMVE, whereas marked injury occurred to bovine aortic endothelial cells included as positive controls. When PMN were added to the milieu, significant EMVE injury occurred. Injury to EMVE was not diffuse, but was restricted to areas of PMN contact; intervening zones of EMVE were normal. The potential involvement of PMN-derived toxic oxygen intermediates was examined by measuring superoxide anion (O_2^-) generation. PMN exposed to LPS for 1 hour generated from 22% to 55% more O_2^-, depending on the agonist used and concentration of LPS. In this *in vitro* model of endotoxin-mediated damage to microvascular endothelium, LPS-induced injury was PMN-dependent, and LPS caused no direct effects. In addition, the mechanism of PMN-dependent injury involved close PMN-endothelial proximity or contact since endothelial cells not in contact with PMN remained uninjured. LPS-induced enhancement of PMN reactive oxygen intermediate generation may be an important mechanism in the mediation of the microvascular endothelial cell injury. (Supported by a Zweig Memorial Fund Grant)

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RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (RH-GM-CSF) PRIMES AND RECRUITS RHESUS MONKEY NEUTROPHIL (PMN) RESPONSES TO N-FORMYL-L-METHIONYL-L-LEUCYL-L-PHENYLALANINE (FMLP). D.M. Linnekin, R.L. Monroy, G. Murano, R.E. Donahue and T.J. MacVittie (Spon: M.L. Patchen). Armed Forces Radiobiology Research Institute, Bethesda, MD, 20814-5145.

RhGM-CSF has been shown to increase production of superoxide anion elicited by FMLP in human PMNs. Recent reports indicate that in the human, populations of PMNs nonresponsive to FMLP can be recruited to a FMLP responsive state by rhGM-CSF pretreatment. The purpose of these studies were to determine the effects of rhGM-CSF on FMLP induced motility and oxidative metabolism in the rhesus monkey and to discern if rhGM-CSF enhances the oxidative metabolism of those FMLP responsive populations (priming) as well as convert nonresponsive populations to a responsive state (recruitment). PMNs were obtained by ammonium chloride lysis of peripheral blood drawn from sedated monkeys (ketamine hydrochloride, 10 mg/kg, i.m.). PMN motility was evaluated counting numbers of cells migrating to FMLP (.1 uM) through the 5 uM pores of polycarbonate filters in a 48 well chemotactic chamber. Generation of the respiratory burst was determined by assessment of formazan deposition resulting from nitroblue tetrazolium (NBT) reduction after 15' exposure to FMLP (.1 uM). Pretreatment of rhesus monkey PMNs with 10 and 100 U/ml of rhGM-CSF for 5' *in vitro* increased the numbers of cells migrating to FMLP to 93% and 126% (respectively) over baseline. The percent of PMNs reducing NBT in response to FMLP increased from 20.5% to 78.7% after rhGM-CSF (10 U/ml) pretreatment for 120'. The intensity of NBT positive cells were scored to determine the effect of rhGM-CSF on FMLP responsive PMNs. PMNS pretreated for 120' with rhGM-CSF has a mean intensity score of 4.17 in contrast to the 1.55 score obtained with untreated PMNs. These data demonstrate that rhGM-CSF recruited FMLP nonresponsive PMN populations as well as enhanced the oxidative burst of FMLP responsive PMNs.

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Depression of human granulocyte chemiluminescence with Schistosoma mansoni schistosomulae

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The effect of S. mansoni schistosomulae on human polymorphonuclear leukocyte (PMN) and monocyte phagocytosis was studied. S. mansoni was isolated from a patient at KKUH. Monocyte or PMNs were obtained from control volunteers and inoculated with S. mansoni schistosomulae. Phagocytosis of PMNs and monocyte was measured using Phorbol Myristate Acetate (2ug/ml) soluble stimulant or opsonized zymosan (2.5mg/ml). Results showed that S. mansoni schistosomulae had a profound inhibition on phagocytosis of both PMNs or monocyte. Beside, blood obtained from patient chronically infected with S. mansoni showed a marked inhibition in whole blood phagocytosis. These results indicated the importance of PMNs and monocyte in the pathophysiology of schistosomiasis.

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INHIBITION OF EOSINOPHIL SUPEROXIDE GENERATION BY MAST CELL GRANULES. K. N. Dileepan, K. M. Simpson, S. Lynch, D.J. Stechschulte (Sponsor: Tsuneo Suzuki), Department of Medicine, Univ. of Kansas Medical Center, Kansas City, KS. 66103

We have recently reported (FASEB J. 2: A1452, 1988) that mast cell granules (MCG) are capable of modulating macrophage superoxide (O_2^-) production. MCG effect on macrophage respiratory burst has been attributed to scavenge of O_2^- by the MCG-bound superoxide dismutase (SOD). In the present study we explored MCG effect on eosinophils (EOS) O_2^- production. Peripheral blood was collected from a patient with hypereosinophilic syndrome whose blood leukocyte count was 24,200/mm^3, of which 59% were EOS. EOS were isolated from the leukocytes to 90% purity by metrizamide gradient centrifugation. MCG were isolated from rat serosal mast cells by the established procedure. EOS O_2^- production was determined by the SOD sensitive reduction of cytochrome c with and without phorbol myristate acetate (PMA) activation. In a five minute assay, PMA-activated EOS produced 7.53 nmoles of $O_2^-/10^6$ cells vs 1.11 nmoles/10^6 resting cells. Preincubation of EOS with MCG for 5 minutes prior to activation with PMA resulted in a marked decrease in O_2^--mediated cytochrome c reduction (92%). Addition of MCG to unactivated EOS also caused inhibition (82%) of O_2^- production. Electron microscopic evaluation revealed the presence of MCG in the vacuoles of EOS that had been exposed to MCG. Analysis of MCG has shown that these organelles contain substantial amounts of SOD activity which remain matrix-bound under physiologic conditions. These results suggest that phagocytosis of SOD-rich MCG by EOS results in the rapid scavenge of O_2^- presumably in a similar fashion noted for macrophages. (Supported by Carey Arthritis Fund).

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PROPERTIES OF EOSINOPHIL ADHESION *IN VITRO*. P.J. Koker, C.C. Clarke, R. Rothlein and C.D. Wegner (Spon: A.S. Rosenthal). Departments of Pharmacology and Immunology, Boehringer Ingelheim Pharma. Inc., Ridgefield, CT 06877.

Eosinophils have been reported to play an important pathologic role in parasite infections, hypersensitivity diseases and asthma. While eosinophil chemotaxis and activation have been studied extensively, eosinophil adhesion (also required for migration and cytotoxicity) has not. The purpose of this study was to investigate the adhesive properties of eosinophils *in vitro*. Cynomolgus monkey (*Macaca fascicularis*) tissue (lung) eosinophils were obtained by bronchoalveolar lavage and purified on a continuous density gradient of Percoll.

Final purity was > 93%. After washing, the eosinophils were added to 96 well flat bottomed plastic tissue culture plates (5 x 10^3 cells/well). After a 90 minute incubation at 37°C, the non-adhered cells were removed by an automated plate washer. Adhered cells were quantitated visually and by performing a colorimetric assay for eosinophil peroxidase (EPO). Eosinophils were found to spontaneously (without the addition of a stimulus) adhere to the bottom of non-protein coated wells but not protein-coated wells. Of the stimuli tested, platelet activating factor (PAF) and solid phase immune complexes (IC) were the most potent stimulators of eosinophil adhesion to protein coated wells. Interestingly, many reported eosinophil chemoattractants (e.g., histamine, LTB_4, ECF-A) as well as reported enhancers of eosinophil activation (e.g., IL-5) did not stimulate eosinophil adhesion (see Table, * mean ± S.E.).

Coated	Stimuli	Conc.	N	EPO units*	% Augment
Protein	None	---	5	260 ± 12	0
None	None	---	6	736 ± 43	180
Protein	PAF	10^{-6} M	6	600 ± 39	130
Protein	IC	---	6	629 ± 88	141
Protein	Histamine	10^{-6} M	6	254 ± 15	-2
Protein	LTB_4	10^{-6} M	6	150 ± 24	-42
Protein	ECF-A	10^{-6} M	6	213 ± 10	-18
Protein	IL-5	100 units	6	262 ± 33	1

We conclude that eosinophil adhesion is regulated independently of eosinophil chemotaxis and/or activation.

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MODULATION OF HEMATOPOIESIS IN THE GOLDEN SYRIAN HAMSTER BY THE ENDOCRINE SYSTEM. J.A. Hightower, M.J. Horacek, M.O. Dada and C.A. Blake. Sch. Med., Univ. South Carolina, Columbia, SC 29208.

Complex interactions occur between elements of the immune system and the endocrine system. While performing experiments to test the effects of various hormones on transplanted pituitary glands, we examined the effects of these hormones on a variety of hematological parameters in intact hamsters (9- or 14-wk-old) and in 14-wk-old hamsters either orchidectomized (ORCH) or orchidectomized and hypophysectomized (ORCH-HYPOX) 5 wks previously. Each ORCH-HYPOX hamster had a pituitary gland allografted beneath its renal capsule and was treated with water, estrogen (E), thyrotropin releasing hormone (TRH), somatostatin, insulin-like growth factor I, melatonin, or VIP for 16 days. TRH increased the thymus weight/body weight (BW) and the eosinophil (PME) count, but decreased the numbers of circulating large mononuclear cells. E also increased the PME count and decreased the hematocrit. The other hormones did not affect any of the following hematological parameters: numbers of thymocytes, splenocytes, platelets, erythrocytes, reticulocytes, and femoral bone marrow cells; weights of thymus glands and spleens; hematocrits, and peripheral blood leukocyte differentials. We noted an age-associated reduction in thymus weight which was unaccompanied by a reduction in the number of thymocytes/mg thymus. Orchidectomy prevented the age-associated reduction in absolute thymus weight and partially prevented the age-associated reduction in thymus weight/BW. This study suggests that TRH and E modulate hematopoiesis. Whether these two hormones act directly, or indirectly through the pituitary, and whether their similar effect on the PME count is related to their ability to release prolactin, remains to be determined. Supported by NIH grant HD22687.

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INVOLVEMENT OF MONOCYTES IN SELECTIVE PRODUCTION OF EOSINOPHIL CHEMOTACTIC LYMPHOKINE. M. Hirashima. Kumamoto Univ. Med. Sch., Kumamoto, Japan.

OKT4-positive T cells from patients with hypereosinophilic syndrome produce a lymphokine chemotactic for eosinophils (ECL) without any additional stimulation, such as antigen or mitogen. This spontaneous ECL production is induced by a monocyte- or macrophage-derived factor (MDF) with molecular weight of about 70,000 to 100,000 from the patients, because T cells from normal donors become to produce ECL activity when they are previously treated with MDF. MDF-treated T cells produce little or no chemotactic activities for neutrophils and macrophages.

In contrast, T cells from patients infected with *Schistosoma mansoni* produce ECL activity when they are stimulated with 1 ug/ml Con A, although those from normal donors fail to produce ECL activity by the stimulation. Normal T cells, however, selectively produce ECL activity (but not chemotactic activities for neutrophils and macrophages) by Con A (1 ug) stimulation if they were pretreated with monocyte supernatants from the patients. Monocyte supernatants from the patients, unlike MDF, fail to induce ECL production. T cells require simultaneous Con A stimulation to produce ECL activity. The potentiating activity for ECL production (ECF-PF) is mainly recovered from molecular range between 45,000 and 70,000.

Present data indicate that there are at least two different mechanisms for ECL production, and that monocyte-derived factors (MDF and ECF-PF) may involve ECL production. Comparative study between two ECL activities, and that between MDF and ECF-PF will be presented.

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FORMALIN-FIXED MACROPHAGES BIND TUMOR TARGETS SIMILARLY TO VIABLE MACROPHAGES. Stephen Keith Chapes Division of Biology, Kansas State University, Manhattan, KS 66506.

Macrophage killing of tumor cells can occur via several mechanisms. Tumor cells that are not sensitive to the cytotoxic effects of tumor necrosis factor still can be killed by a contact-dependent mechanism. One of the most important steps during contact-dependent killing is the recognition or binding of tumor cells. One way to measure whether recognition occurs is to use binding procedures that use 1300 x g centrifugal force to disrupt macrophage-tumor cell conjugates; those conjugates that remain together are believed to be specifically bound. During studies attempting to better understand macrophage recognition of tumor cells, we determined that macrophages fixed with 1% formalin in PBS would bind targets similarly to viable macrophages. Peritoneal macrophages from C3H.OL mice, activated with P. acnes were used in these experiments. Like binding between viable macrophages and tumor cells, the process was temperature dependent. Targets only bound to macrophages at 37°C. The process was Ca^{++}-dependent. Binding did not occur in the presence of EDTA. Fixed macrophages were able to discriminate targets similarly to viable macrophages. Targets not bound by viable macrophages, like spleen cells, F5m tumor cells or VERO cells were not bound by fixed macrophages, whereas other tumor cells (e.g. F5b) were bound. We also found that target cells could not be fixed in 1% formalin for binding to occur. These data suggest that the receptor on the macrophages for tumor cell recognition is functional in the absence of active physiological processes. In contrast, tumor cell processes that are dependent upon target cell viability are required for binding. These studies were supported by National Cancer Institute Grant CA40477 and Bioserve Space Technologies NASA contract #NAGW-1197.

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REGULATION OF TUMOR-INDUCED MYELOPOIESIS AND THE ASSOCIATED IMMUNE SUPPRESSOR CELLS IN MICE BEARING METASTATIC LEWIS LUNG CARCINOMAS BY PROSTAGLANDIN E_2. M.R.Young, M.E.Young and K.Kim Research Serv., Hines V.A. Hosp., Hines, IL 60141 and Dept. Pathology, Loyola Univ. Stritch Sch. Med., Maywood, IL 60153

The in vivo and in vitro effects of prostaglandin E_2 (PGE_2) and of its stable analog, 16,16-dimethyl-PGE_2 ($dmPGE_2$), on myelopoiesis and immune parameters of mice bearing metastatic variant Lewis lung carcinoma (LLC-C3) tumors were assessed. In vitro studies showed that PGE_2 reduced the growth of myeloid progenitor cells (CFU) and reduced the generation of bone marrow immune suppressor cells in co-cultures with LLC-C3 supernatants. In vivo studies showed that 4 daily injections of $dmPGE_2$ into LLC-C3 tumor-bearing mice caused some reduction in femoral bone marrow CFU and had an insignificant effect on bone marrow suppressor cell activity. In contrast, spleen cells of $dmPGE_2$-treated LLC-C3-bearing mice showed a reduction in cellularity, CFU, and the level of spontaneous proliferation; a reduction in suppressor cell activity; and an increase in blastogenesis. Thus, short-term $dmPGE_2$ treatment of LLC-C3-bearing mice was beneficial, particularly with regard to the spleen, as it limited the tumor-induced hematopoietic stimulation and reduced the associated immune suppressor cell activity. (Supported by the Medical Research Service of the V.A. and by Grant CA-45080 from the National Institutes of Health)

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PHENOTYPIC DIFFERENCES BETWEEN NORMAL AND TUMOR-BEARING HOST MACROPHAGES. A.D. Yurochko, R.H. Pyle, and K.D. Elgert. Dept. of Biology, Microbiology and Immunology Section, and Veterinary Biosciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

As a tumor grows, changes occur in the function and phenotype of macrophages (MØ). To chart the course of phenotypic changes during tumor growth, MØ from tumor-bearing hosts (TBH) were collected at different time points (0, 7, 14, and 21 days post tumor cell inoculation). These MØ were labeled with fluorescently-tagged monoclonal antibodies against the MØ surface antigens Mac-1, -2, -3, and Ia. Flow cytometry showed that a tumor-induced shift of these antigens occurred in peritoneal and splenic MØ. During tumor growth, the total number of peritoneal MØ bearing Mac-2, -3, and Ia markers decreased (23%, 18%, and 73%, respectively), while Mac-1$^+$ MØ remain unchanged. By analyzing the data on a two dimensional histogram and comparing the size of MØ to cell surface antigen expression, we identified distinct subpopulations of peritoneal MØ. The three distinct size versus antigen expression subpopulations were about 10-13, 14-17, and 18-22 microns for the small-, medium-, and large-sized populations, respectively. The number of large-sized MØ bearing the Mac-1 and -2 antigen decreased (37% and 38%), while the number of large-sized MØ bearing the Mac-3 antigen did not decrease during tumor growth. Medium-sized MØ expressing the Mac-3 antigen decreased 33% during the tumor growth, while no differences were seen in the number of medium-sized MØ bearing the Mac-1 or -2 antigens. Concomitant with the decrease in large-sized MØ bearing Mac-1 was an increase in the number of small-sized MØ expressing this antigen. Peritoneal MØ displaying Ia antigen were mostly small-sized (4-7 fold increase over the medium-sized and none in the large-sized population). Their Ia antigen was nearly absent by 21 days of tumor growth, with ~ 4% of the cells labeling positive (a 73% drop from normal host MØ). In splenic MØ, the number of cells expressing Mac-1 increased by 116% during tumor growth, while Mac-2 and Mac-3 bearing cells increased 43% and 64%, respectively. Additionally, splenic Ia$^+$ MØ decreased by 36% during tumor growth, with a concomitant decrease in Ia antigen expression per cell. Unlike the peritoneal MØ, the splenic MØ did not show distinct size versus antigen expression subpopulations. Tumor growth, however, did alter the size range of the TBH MØ. Normal host MØ ranged from about 8-20 microns, while TBH MØ were more homogeneous and ranged from about 8-13 microns. These data suggest that MØ from different anatomical sites are phenotypically different and that tumor growth modulates phenotypic alterations in peritoneal and splenic MØ subpopulations. Combined with our previous work, this suggests a relationship between changes in MØ phenotype and tumor-induced dysfunction of MØ-mediated immune activity. (Research supported by an NIH Biomedical Research Support Grant and a Sigma Xi Grant.)

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INHIBITION OF TUMOR CELL GLUTAMINE UPTAKE AS AN INDICATOR OF BOTH OXIDATIVE AND NON-OXIDATIVE CYTOTOXICITY CAUSED BY STIMULATED NEUTROPHILS. Douglas B. Learn and Edwin L. Thomas. Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101

Inhibition of glutamine uptake by CCRF-CEM tumor cells exposed to stimulated neutrophils revealed both an oxidative and non-oxidative portion of cytotoxicity. When tumor cells were incubated in suspension with neutrophils, inhibition of tumor cell glutamine uptake was mediated by the neutrophil myeloperoxidase/hydrogen peroxide/chloride system. Superoxide dismutase and exogenous myeloperoxidase potentiated the damage, and inhibition was blocked by the addition of catalase, an inhibitor of myeloperoxidase, or compounds that scavenge hypochlorous acid or chloramines. When cells were incubated as a pellet, a portion of the inhibition could not be blocked in this way, indicating that a non-oxidative mechanism contributed to inhibition under these conditions. This non-oxidative inhibition was not apparent when cells were incubated as a suspension. Inhibition was obtained under conditions that did not result in release of ^{51}Cr from tumor cells labeled with [^{51}Cr]-chromate, indicating that inhibition of glutamine uptake measured cytotoxicity rather than cytolysis. Inhibition of glutamine uptake was rapid and was obtained at effector:target cell ratios as low as 0.5:1. CCRF-CEM tumor cell glutamine uptake was characterized and found to be 64% by way of the sodium-dependent A and ASC systems, with the remainder being by way of the sodium-independent L system. The rate of uptake was a stable parameter and did not respond rapidly to the presence or absence of energy sources. The results indicate that stimulated neutrophils are potent antitumor effector cells when cytotoxicity rather than cytolysis is the measure of activity. Amino acid and other transport systems may be significant targets for cytotoxicity caused by stimulated neutrophils and other effector cells. Because glutamine is required for growth of many tumor cells, inhibition of glutamine uptake may represent a significant tumoristatic or tumoricidal effect.

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A COMPARISON OF LEUKOCYTE INFILTRATON INTO AN IMMUNOGENIC AND A NONIMMUNOGENIC MURINE TUMOR. R.A. Sneed, A.P. Stevenson, C.C. Stewart. Cell Biology Group, Los Alamos National Laboratory, Los Alamos, N.M. 87545.

Using concomitant immunity as an endpoint, we found that BALB/c mice injected with 10^5 EMT6 tumor cells could completely reject a secondary challenge by 10 days. In contrast, when given a primary injection of 10^5 Colon 26 cells, mice could never reject any challenge dose of Colon 26. We excised primary tumors in exponential growth and determined the leukocyte content using multiparameter flow cytometry. Tumors were dispersed, and cells were stained with Hoechst 33342 (for DNA content) and with FITC-Thy1 and phycoerythrin-Mac1 to identify T cells, macrophages, and granulocytes. It was found that approximately two times as many leukocytes were in EMT6 tumors as compared to the Colon 26 tumors. While no Thy1$^+$ EMT6 cells were found, ~50% of Colon 26 tumor cells were Thy1$^+$. In the EMT6 tumor, macrohpages were in cell cycle, but, in the Colon 26 tumor, T cells were in cell cycle. Thus, profound differences were found between the leukocyte infiltrates of the two tumors.

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THE EFFECT OF SELENIUM ON THE TUMORCYTOTOXICITY OF MOUSE PERITONEAL MACROPHAGES AND SPLEEN LYMPHOCYTES.
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Selenium (Se) has been shown to influence the development and expression of various biological processes. This study examined the ability of Se to modulate the tumorcytotoxicity of peritoneal exudate macrophages (PEC) activated in vivo, and spleen lymphocytes activated in vitro. PEC were collected from 14-week old, male C57Bl/6J mice maintained for 8 weeks on Se-deficient (0.02 ppm Se), normal (0.2 ppm Se), or Se-supplemented (2 ppm Se) Torula Yeast diets. Immune macrophages were stimulated by i.p. injection with 5×10^6 P815 cells ten days prior to PBC collection. The non-adherent PEC were removed, and 2×10^5 macrophages were cocultured for 16h with 2×10^4 ^{51}Cr-labeled, freshly isolated P815 cells. The results indicated that Se supplementation of the diet significantly increases, while dietary Se deficiency significantly inhibits the ability of peritoneal macrophages to destroy tumor cells specifically. Cytotoxic spleen lymphocytes from animals maintained on normal diet were generated by coculture for 5 days with mitomycin C-treated P815 cells (50:1 ratio) in culture medium supplemented with 1×10^{-9}M to 1×10^{-6}M Se (as sodium selenite). The final endogenous level of Se in the medium contributed by 5% foetal calf serum was determined as 6.33×10^{-9}M. The ability of the lymphocytes to destroy P815 cells was studied with the 4-h-^{51}Cr release-assay. Se supplementation, i.e., 5×10^{-9} to 5×10^{-7}M, resulted in a significant reduction in the number of lymphocytes required to destroy a fixed number of target cells. Thus, modulation of Se in the cellular environment may alter the ability of a host to destroy tumors. (Supported by AICR Grant #86A08R87B)

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DEPRESSED CELL MEDIATED IMMUNITY IN PATIENTS WITH SEVERE INHERITED FORMS OF EPIDERMOLYSIS BULLOSA.
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Epidermolysis bullosa (EB) refers to a group of genetic diseases characterized by marked skin fragility and blister formation. Patients with severe forms of EB have a high incidence of skin cancers. Peripheral blood mononuclear cells (PBMC) were obtained from twenty-six EB patients and from an equal number of age matched controls. The PBMCs were evaluated for histocompatibility antigens, surface antigen expression, natural killer cell activity and lymphokine production. Of the twenty-six patients examined, eight had EB simplex (EBS), six had junctional EB (JEB), three had dominant dystrophic EB (EBDD), and eight patients had recessive dystrophic EB (EBDR). Natural killer cell activity was markedly depressed in EBDR and EBDD patients and was not significantly affected in EBS and JEB patients as compared to controls. There was a decrease in interleukin-2 production (assayed by CTLL-bioassay and ELISA) and interleukin-1 production (assayed by RIA) in the four groups of EB patients in comparison to normal controls. In vitro production of interferon-γ but not interferon-α was markedly decreased in the EB patients. In vitro production of tumor necrosis factor (TNF) was also suppressed in EBDD, EBDR, and JEB patients, but not in EBS patients. Evaluation of histocompatibility antigens revealed HLA-DR4 homozygosity in six of seven patients with EBDR. The EBDR patients had significantly depressed helper/suppressor (T4/T8) ratios in their PBMCs associated with a marked increase in suppressor T cells. In conclusion, the EB patients showed a decreased natural killer cell activity associated with a decrease in monokine and lymphokine production and an increase in the number of suppressor T cells, reflecting a general reduction of cellular immunity.

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THE SIGNIFICANCE OF FREE RADICAL AND FREE RADICAL SCAVENGERS IN L1210 LEUKEMIA.
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L1210 leukemia is a murine leukemia which is associated with anemia and marked neutrophilia. In order to determine the significance of free radicals (FR) in this disorder, we determined the presence and localization of free radical scavengers (FRS) and scavenger-like systems in L1210 leukemia cells obtained in vivo and from in vitro cultures. FR are metabolized or detoxified by certain FRS such as glutathione (GSH and GSSG), superoxide dismutase (SOD) and enzymes such as epoxide hydrolase (EH). In all cases specific fractions of L1210 cells, bone marrow and liver were examined for FR/FRS levels. Reduced (GSH) and oxidized (GSSG) glutathione were measured fluorometrically using O-ophthalaldehyde (OPT). SOD was determined colorimetrically utilizing pyrogallol by substrate autolysis inhibition, and EH was determined by utilizing [^{3}H]styrene oxide as a substrate. Ratios of GSH/GSSG in fractions prepared from in vivo and in vitro L1210 cells showed a predominance of GSH-reductase with the highest activity in mitochondria (ratio = 15 vs. 10). Normal liver showed a similar pattern, whereas leukemic liver showed altered GSH/GSSG ratios in mitochondria and microsomes. Leukemic bone marrow showed a predominance of GSH-reductase in all fractions. EH activity was highest in microsomal fractions obtained from L1210 cells grown in vitro and found to become increased in both the mitochondrial (100%) and microsomal (200%) fractions when cells were exposed to retinoic acid (RA) in culture. SOD activity in the cytosolic (21.2 U SOD/mg) and mitochondrial (12 U SOD/mg) fractions, whereas leukemic liver showed a significant decrease in activity in all fractions compared to normals. SOD was determined in fractions taken from L1210 cells in vivo and in vitro. Results from these studies indicate that certain FRS systems are functional in L1210 leukemic animals. Furthermore, variations in the ratios or levels may be of significance in the leukemic and hematological states.

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MODULATION OF MACROPHAGE-TUMOR CELL CYTOTOXIC INTERACTIONS BY HYPERTHERMIA.
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Hyperthermia in the therapeutic (≥ 42°-43°C) and febrile (≤ 39°-40.5°C) ranges modulated the cytotoxic activities of monocytes (MO)/macrophages (MØ) and their monokines against tumor cells. These modulatory interactions displayed striking treatment sequence dependencies and in some sequences markedly enhanced cytotoxic activities could be realized. Both BCG-activated MØ and human peripheral blood MO showed better retention or pronounced augmentation of their ability to secrete the cytotoxic monokines TNF, respiratory inhibition factor (RIF) and iron-releasing factor (FcRF) if endotoxin-triggering preceded 1 hr heating at 42° or 43°C by 1.5 to 4 hr than if the same heating was concomittant with or preceded triggering by 1 to 4 hr. Qualitatively similar observations were made when 24 hr heating at 39° or 40.5°C preceded or was concomittant with triggering. Studies of MO and MØ in coculture with various tumor cells also indicated that endotoxin-triggering prior to heating retained or enhanced function, whereas heating simultaneous with triggering significantly inhibited effector cell ability to deliver lethal hits. Profound treatment sequence

dependencies were also seen when addition of monokines to tumor cells was varied about the heat treatment. Sensitization to TNF of L929 and TNF resistant EMT-6 tumor cells was up to 10,000-fold if monokine administration preceded 1 hr heating at 43°C, whereas the reverse halved or eliminated sensitization, respectively. Augmentation or retention of RIF and FcRF toxicities, respectively, followed a similar pattern in EMT-6 cells. Both tumor cell types were also sensitized to TNF (but not RIF or FcRF) if treatment preceded 24 hr heating at 40.5°C. These results support the hypothesis that appropriately constructed sequences for MO/MØ priming/triggering or monokine treatment of tumor cells combined with hyperthermia can significantly potentiate the cytotoxic interactions between effector cells or exogenously added monokines and their tumor cell targets. (ACS IM-419 to JK and USPHS CA32745 to SPT).

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ROLE OF ANTIBODY IN COMPLEMENT-MEDIATED KILLING OF TRITRICHOMONAS FOETUS. M. K. Aydintug, P. R. Widders (Spon: S.M. Taylor). Washington State Univ., College of Veterinary Medicine, Pullman, WA. 99164-7040.

Tritrichomonas foetus is a venereal pathogen of cattle, causing infertility, abortion and pyometritis. Persistence of the organism in the reproductive tract of cows and bulls implies that T. foetus is capable of evading host defenses in the bovine genital tract. In this study, antibody-dependent and -independent complement (C) killing of T. foetus was measured using an assay of trichomonad viability based on protozoal uptake of tritiated adenine. Trichomonads were grown in Diamond's medium, washed and resuspended in phosphate buffered saline (PBS) containing 20 mM glucose (G) and 1% fetal bovine serum (FBS). To measure killing, $5x10^5$ trichomonads were incubated at 37°C for 30 minutes with 150 µl of test serum (in the absence or presence of antibody to T. foetus). At the end of the incubation, reaction mixtures were pulsed with tritiated adenine in PBS-G-FBS, incubated for 60 minutes at 37°C, and the reaction stopped by the addition of formaldehyde. The labeled suspension was harvested onto glass fibre filters and counted in scintillant. Killing was measured in the presence of complement-preserved hypogammaglobulinemic bovine serum, using heat-inactivated serum as control. The role of antibody in promoting complement-mediated killing was measured using hyperimmune bovine serum. Moderate levels of killing were measured in the absence of antibody only with high concentrations of complement-preserved serum. However, very low concentrations of hyperimmune serum promoted significant enhancement of complement killing. Treating complement with Mg-EGTA did not abolish antibody-dependent or -independent killing of T. foetus. These results demonstrate antibody-dependent enhancement of complement killing of T. foetus, and suggest that killing is independent of the classical pathway of complement activation.

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CORRELATION OF VIRULENCE IN VIVO, SUSCEPTIBILITY TO KILLING BY MURINE POLYMORPHONUCLEAR NEUTROPHILS (PMN) IN VITRO, AND PMN SUPEROXIDE ANION (O_2^-) INDUCTION IN BLASTOMYCES DERMATITIDIS (BD) ISOLATES. C.J.Morrison* and D.A.Stevens. Inst. Med. Res., Santa Clara Vly. Med. Ctr., San Jose, CA 95128 and Stanford U., Stanford, CA 95304.

We previously found a correlation between in vitro fungal killing by PMN and in vivo virulence for two isolates of BD, V and A2, whether PMN were obtained from the peripheral blood (PB-PMN) of normal mice or from the peritoneal cavity (PEC) of BD immune mice given killed BD i.p. 24 h earlier. Four other isolates of varying virulence were compared for susceptibility to PMN killing and the ability to induce O_2^- production by PB-PMN in vitro. A BD isolate of low virulence (GA-1) was most susceptible to killing by PB-PMN (97.5 ± 1.0% dead) or PEC (90.3 ± 7.5%). Highly virulent isolates, V and V40, were significantly less susceptible to in vitro killing by PB-PMN (38.0 ± 14.9%, 42.3 ± 19.3%, respectively) or PEC (51.7 ± 12.4%, 62.1 ± 16.1%). Low virulence BD isolates, A2 and KL-1, were intermediate in susceptibility to PB-PMN (69.1 ± 13.3%, 68.4 ± 15.1%, respectively) or PEC killing (80.2 ± 12.7%, 85.6 ± 12.6%). V and V40 were poor inducers of O_2^- production by PB-PMN (V: 1.2 ± 1.6 nmol/10^6PMN/h; V40: 2.0 ± 2.0 nmol/10^6PMN/h) while A2, GA-1, and KL-1 stimulated the production of 26.4 ± 9.6, 15.0 ± 5.8, and 11.2 ± 7.2 nmol/10^6PMN/h, respectively. Another low virulence isolate (A), although a good inducer of O_2^- production in vitro (23.6 ± 7.2 nmol/10^6PMN/h), was no more susceptible to in vitro killing by PB-PMN (47.7 ± 15.0%) or PEC (58.9 ± 17.3%) than V or V40. These data indicate that while in vitro killing by PMN and O_2^- induction correlates with virulence for five BD isolates, isolate A provides an exception which may indicate a unique resistance to killing by PMN-generated O_2^-.

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EFFECT OF IONIZING RADIATION ON THE ABILITY OF MURINE PERITONEAL CELLS TO PHAGOCYTIZE KLEBSIELLA PNEUMONIAE. D. G. McChesney, G.S. Madonna and G.D. Ledney (Spon: M. Patchen). Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Exposure of B6D2F1 female mice to ^{60}Cobalt ionizing radiation greatly increases their susceptibility to Klebsiella pneumoniae infection 4 days after irradiation (non-irradiated LD_{50} = 10^7, irradiated LD_{50} = 10^3). By day 4 post radiation the mouse is severely lymphocytopenic and must depend on the nonspecific elements of the immune system (e.g. radioresistant macrophages) to combat infection. One explanation for the increase in susceptibility of these animals to bacterial challenge is that macrophages are no longer able to kill the K. pneumoniae. When the immunomodulator trehalose dimycolate (TDM), known to be a potent macrophage activator, is given i.p. as an oil-in-water emulsion (TDM/O), mice are more resistant to K. pneumoniae infection. This suggests that radiation is affecting the macrophages either directly or indirectly and that TDM/O is capable of altering this effect. Murine resident peritoneal cells obtain 4, 7, or 11 days post 7.0 Gy radiation maintain their ability to phagocytize and kill K. pneumoniae. This killing of K. pneumoniae by this cell population, which is greater than 99% monocytes/macrophages, is enhanced over that of peritoneal cells obtain from non-irradiated mice and is complement dependent. The incubation of TDM/O in vitro with peritoneal cells obtained from normal or irradiated mice causes a slight increase (2-8%) in the ability of the peritoneal cells to phagocytize and kill K. pneumoniae. The percent increase in killing is greater for the cells obtained from non-irradiated animals than from irradiated animals. These results indicate that radiation, in the short term, does not directly affect the ability of the macrophages to phagocytize and kill K. pneumoniae.

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MITIGATION OF THE LETHAL EFFECTS OF IONIZING RADIATION BY 6,6' TREHALOSE DIESTERS. G.S. Madonna, M.L. Patchen and G.D. Ledney. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

Exposure to whole body ionizing radiation results in hematopoietic stem cell depletion, depletion of mature hematopoietic and immune cells, impairment of antimicrobial immunity, and death due to either acquired infections or translocated endogenous intestinal microorganisms. We previously showed that survival of mice exposed to either a lethal dose of radiation or a sublethal dose of radiation plus a lethal bacterial challenge, is increased if they are injected with an oil-in-water

emulsion of the mycobacterial cell wall-derived immunomodulator trehalose dimycolate (TDM), a potent macrophage activator. This suggested that TDM and perhaps other 6,6' trehalose diesters could be potent stimulators of hematopoiesis and nonspecific resistance to infection in immunocompromised hosts. In particular, we compared oil emulsions of TDM (TDM/O) and saline suspensions of a synthetic trehalose 6,6' diester of corynomycolic acid (synTDM) for their ability to, 1) increase survival in lethally irradiated mice, 2) increase splenic endogenous colony forming units (E-CFU, an indicator of endogenous hematopoietic stem cell proliferation), and 3) increase survival in sublethally irradiated, granulocytopenic mice challenged with Klebsiella pneumoniae. TDM/O or synTDM enhanced 30 day survival of C3H/HeN or B6D2F1 female mice when they were injected ip 20h before or 1h after lethal exposure to 60Co radiation. For example, survival of B6D2F1 mice injected with TDM/O or synTDM (100 ug/mouse) 20h before receiving 10.25 Gy was 100% and 90% respectively, whereas only 10% of untreated mice survived. In C3H/HeN mice, injection with TDM/O or synTDM before or after irradiation significantly increased spleen weights and E-CFU counts (day 12 E-CFU) in a dose dependent fashion. For example, the average number of E-CFU per spleen for mice injected with synTDM (800, 400, or 200 ug/mouse) 1h post irradiation were 17.7, 14.9 and 10.5, respectively; untreated = 1.9. Post radiation injection with TDM/O or synTDM also significantly increased 30 day survival of B6D2F1 mice exposed to sublethal radiation and lethally challenged with K. pneumoniae 4 or 14d post radiation (e.g., synTDM treatment = 90% survival, untreated = 0%. These results indicate that in mice immunocompromised by radiation, both TDM/O and synTDM are potent stimulants of hematopoisis and nonspecific resistance to infection and underscore their prophylactic, as well as therapeutic potential in the treatment of infections in various immunocompromised conditions.

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PHAGOCITOSIS E MYCOBACTERIA BY CULTURED-HUMAN MACROPHAGES. M.Arango, F. Marinalde, L.F. Barrera, L.F. Garcia Univ. de Antioquia, Medellin, Colombia.

With the aim of studying the interactions between mycobacteria and human macrophages, we tried to standardize a technique to measure phagocitosis and activation in human monocyte-derived macrophages.

The hanging-drop technic was used to measure phagocitosis and intracellular replication of Mycobacterium tuberculosis (H37Rv and H37Ra) and Mycobacterium bovis (BCG). In cultured macrophages, either activated or non-activated with supernatants from lymphocyte cultures stimulated with PPD or PHA there were no significant differences in the amount of bacteria phagocytized by macrophages from skin-positive or skin-negative individuals. However, the lymphocyte supernatant from skin-positive subjects induced a significant activation of macrophages resulting in a reduction of infected cells and in the number of acid fast bacilli (AFB)/macrophage.

In order to obtain a more precise measurement of these effects we used 3H-Uracil incorporation. Macrophages were incubated with various concentrations of mycobacterias. A relation 1:1 between the number of bacteria and 3H-Uracil incorporation was demonstrated. The replication seemed to depend on a ratio of Mycobacteria/macrophage equal or higher than 2/1. H37Rv exhibited the best intracellular growth. With this technique it was found that supernatant from PHA stimulated lymphocytes reduced significantly the incorporation of 3H-Uracil, reflecting activation of macrophages and reduction of bacterial intracellular growth, when compared with non-supernatant stimulated macrophages. Both technics indicated that there is much individual variation which may be probably due to genetic differences. To avoid this variation we are in the process of evaluating the myelomonocytic line U937 for its possible use as a model for the study of interactions between mycobacteria and the immune system. (Supported by grant from WHO Geneva and Colciencias).

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THE RESTORATIVE EFFECTS OF GAMMA INTERFERON AND CLOFAZAMINE ON PHAGOCYTE DYSFUNCTION CAUSED BY A 25 KILODALTON FRACTION FROM *MYCOBACTERIUM TUBERCULOSIS*. A.A. Wadee, A.R. Rabson and R. Anderson (Spon. J. Metz). Dept. of Immunology, School of Pathology of the South African Institute for Medical Research and the University of the Witwatersrand, Johannesburg, 2000, Republic of South Africa.

We have previously demonstrated that a 25-kDa fraction from *Mycobacterium tuberculosis* inhibits the intracellular killing ability of phagocytes. The present study undertook to examine the interference of a variety of phagocytic functions associated with anti-microbial activity by this mycobacterial component. Our studies demonstrate that the 25-kDa mycobacterial fraction inhibited the ability of both polymorphonuclear (PMN) leukocytes and cultured monocytes to release lysosyme and produce hydrogen peroxide (H_2O_2). In addition this mycobacterial fraction reduced hexose monophosphate shunt (HMPS) activity. Because both gamma interferon (IFN-γ) and clofazamine are associated with increasing phagocyte microbicidal activities, the present study undertook to examine their role in reversing the inhibitory effect of the 25-kDa fraction. Both IFN-γ and clofazamine at a variety of concentrations were capable of partially reversing the inhibitory effects of the mycobacterial components in all of the above systems. These studies indicate important mechanisms in the understanding of the pathogenesis of tuberculosis and suggests that IFN-γ and clofazamine may have a therapeutic role in mycobacterial diseases.

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BOVINE POLYMORPHONUCLEAR NEUTROPHILLIC GRANULOCYTE-PRODUCT WITH ANTIVIRAL (INTERFERON-LIKE) ACTIVITY: CHARACTERIZATION OF THE INDUCTION, SECRETION AND ACTIVITY OF "POLYFERON". H. Bielefeldt Ohmann, M. Campos, D. Fitzpatrick, M.J.P. Lawman & L.A. Babiuk. Veterinary Infectious Disease Organization, 124 Veterinary Road, Saskatoon, Saskatchewan S7N 0W0, Canada.

The potential importance of bovine polymorphonuclear neturophils (PMN) in the antiviral defence against herpesvirus infection has been amply demonstrated. In addition to their phagocytic and cytotoxic activities, a key function of the PMNs may be the production of an antiviral substance with interferon-like activity. The product, for which the name "polyferon" (PF) was coined, is produced within 12-18 hours upon direct encounter of the PMN with bovine herpesvirus type 1 (BHV-1) infected cells, or membranes thereof. Exposure to purified virus does not induce PF. The intimate interaction between PMN and the membranes of virus infected cells was also revealed by EM studies. Bovine cells infected with herpes simplex virus type 1 (HSV-1) could similarly induce PF-production by bovine PMN, whereas cells infected with BHV-2, HSV-2, equine herpesvirus type 1 (EHV-1), bovine respiratory syncytial virus (BRSV), bovine viral diarrhoea virus (BVDV) or parainfluenza type 3 virus (PI-3) were unable to do so. Preliminary experiments suggested that gpIII of BHV-1 might be involved in the induction of PF. However, gpIII transfected murine L-cells, L-cells undergoing an abortive infection with BHV-1 or porcine kidney cells undergoing a productive BHV-1 infection could not induce detectable PF-activity. These results suggest that a complex comprised of both viral product(s) and a host cell factor(s) unique for bovine cells is required for activation of the PMN.

Polyferon is acid labile and cannot be neutralized by antibodies to bovine interferons -alpha$_1$, -beta$_2$, or -gamma. PF may nevertheless belong to the interferon-family of proteins as indicated by its ability to induce 2-5A oligonucleotide synthetase in various cell types, responsive to bovine interferons, and by its antiviral spectrum. To further exclude its similarity to interferon-gamma it was demonstrated that PF cannot induce Ia antigen expression on epithelial and endothelial cells. Whether it could have other biological functions in addition to the strictly antiviral activity is currently being investigated.

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It is generally accepted that effective phagocytosis and killing of intracellular microorganisms require activation of macrophages by antigen-induced limphokines.While the macrophage activating effects of Interferon-γ (IFN) is better defined,the role and its relationship with other limphokines is less understood.In the present work we now report the effect of recombinant Interleukin-2 (IL-2, 1 to 200 U/ml) and IFN (0.1 to 100 U/ml) on the intracellular killing of Listeria monocytogenes by murine proteose-peptone elicited peritoneal macrophages.Macrophages treated with IFN alone developed a good bactericidal activity against L. monocytogenes,while macrophages treated with IL-2 alone failed to develop this effector reaction.However the rate of intracellular killing of L.monocytogenes by IFN-activated macrophages was significantly higher when the cells were precultured with IL-2.Comparable results were obtained when macrophages were treated reversely with IFN followed by IL-2 or simultaneously with both IFN and IL-2. The effect of IL-2 was not overcomed by exogenous Prostaglandin (PG) E2 (up to 10^{-6} M) nor was influenced by addition of endogenous PGE2 inhibitor indometnacin (10^{-5} M),indicating that PG pathway is not involved in this system. The results of these studies indicate that,although IFN plays an important role in macrophage activation to intracellular microorganisms,other limphokines may potentiate its effect by increasing,in presence of IFN,the effector antimicrobial mechanisms of macrophages.The activity of other limphokines is currently under investigation.

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SEPARATE AND COMBINED EFFECTS OF rIL-1α, rTNF-α AND rIFN-γ ON ANTIBACTERIAL RESISTANCE. R. Kurtz, J. Roll and C.J. Czuprynski. Univ. WI Sch. Med., Madison, WI 53706

Our laboratory has reported previously that administration of murine rIL-1α substantially enhanced the resistance of mice to Listeria monocytogenes infection. We have extended these findings and demonstrated that two other cytokines, rTNF-α and rIFN γ, possesses similar abilities to enhance anti listeria resistance. We then addressed the possibility that these cytokines might result in additive or synergistic enhancement of antibacterial resistance. Simultaneous administration of rIL-1α + rIFN-γ enhanced anti listeria resistance to a greater extent then did either cytokine alone, although the modest results did not imply a synergistic interaction between the two cytokines. Maximal protection was observed when IL-1 + IFN γ were administered concomitant with the bacterial challenge and the mice were sacrificed three days post infection, the time at which the bacterial burden peaks. More dramatic effects were observed with combined administration of rTNF α + rIL-1α. A substantial synergistic enhancement of antibacterial resistance was observed when suboptimal doses of both rTNF and rIL 1 were administered together with the listeria challenge. In contrast combined administration of rTNF + rIFN γ did not lead to enhancement of anti listeria resistance. These data suggest that it may be important to assess the potential synergistic activities of cytokines, such as IL 1 and TNF. It is likely that the greatest protection, with the least amount of deleterious effects may be achieved by administration of "cocktails" consisting of two or more cytokines with complementary activities.

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A SELF-LIMITING SEMLIKI FOREST VIRUS INFECTION ACTIVATES MURINE MACROPHAGES. L.-X. Wu, K. Suryanarayana, K.-C. Lee, R.G. Marusyk, and A.A. Salmi. (Spon: F.S. Morahan) Viral Pathogenesis Research Unit, Department of Medical Microbiology and Infectious Diseases, and Department of Immunology, University of Alberta, Edmonton, Alta., Canada T6G 2H7.

Virus infection of monocytes and macrophages may change immunological functions and modulate the resulting immune response. We have earlier shown that Semliki forest virus (SFV), a single stranded RNA virus of the family Togaviridae, enhances the autoimmune disease, experimental allergic encephalomyelitis (EAE) in mice and have shown that the effect is immunologically mediated. We have now shown that SFV caused a self-limiting infection in thioglycollate-elicited peritoneal macrophages (PM) and disturbed the immunological functions of macrophages *in vitro*. Virus RNA synthesis and virus proteins were demonstrated in SFV-infected PMs up to 5 days p.i. by nucleic acid hybridization and immunofluorescence, respectively. A small amount of infectious virus was also released from infected PMs but no virus was detected after 5 days p.i. Since an immature fraction of bone marrow derived macrophages (BMDM) was resistant to SFV infection, and activation of BMDM with rat ConA supernatant had no effect on virus replication, we conclude that SFV infection depends on the differentiation stage but not on the activation stage of macrophages. SFV infection was found to transiently activate PM, and to induce a temporal production of IL-1 in resting macrophages. Class II antigen (Ia) expression was also induced in infected, resting macrophages. We propose that the Ia-inducing effect may be mediated by receptor-ligand like interaction between virus and macrophages. The results suggest that increased production of IL-1 and enhanced Ia expression in macrophages by a self-limiting virus infection may have a significant potentiating effect on autoimmunity. This study was supported by grants from the Multiple Sclerosis Society of Canada and the Alberta Heritage Foundation for Medical Research.

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ISOLATION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) ON RECOMBINANT HUMAN MACROPHAGE COLONY STIMULATING FACTOR (rM-CSF) TREATED HUMAN MONOCYTES: AN EFFICIENT VIRUS DETECTION SYSTEM. H. Husayni, M. S. Meltzer and H. E. Gendelman Walter Reed Army Inst. Res., Washington, DC 20307-5100

Monocyte/macrophages are major target cells for HIV in man. Brain macrophages, Langerhans cells of skin, follicular dendritic cells of lymph nodes and blood monocytes support virus replication throughout the course of HIV infection despite an often vigorous host immune response. We compared monocyte and lymphoblast HIV isolation techniques in 20 patients with or at risk for the acquired immune deficiency syndrome. Replicate aliquots of patient blood mononuclear leukocytes, isolated by ficoll-hypaque gradient centrifugation, were cocultivated with allogeneic rMCSF-treated monocytes or mitogen-stimulated lymphoblasts. Culture fluids were analyzed for viral antigens and reverse transcriptase activity. The frequency of cells expressing HIV-specific mRNA was quantified by *in situ* hybridization on blood leukocytes. HIV was recovered in monocytes for 19/20 (95%) and in lymphoblasts for 9/20 (45%) patients. Virus isolation into rMCSF-treated monocytes was successful in healthy seronegative and seropositive individuals where lymphoblast assays were negative. That these isolates were HIV-1 was confirmed by *in situ* hybridization and radioimmunoprecipitation assays on the infected cells. Isolation of HIV into rMCSF-treated monocytes is a sensitive recovery system that may have particular utility early in the course of virus infection.

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INFECTIBILITY BY THE HUMAN IMMUNODEFICIENCY VIRUS (HIV) OF HUMAN BLOOD-BORN MONOCYTES/MACROPHAGES AND CHARACTERISATION OF INFECTED MONOCYTES/MACROPHAGES. C. Meichsner, H. Rubsamen-Waigmann, R. Andreesen, M. Limbert, E. Schrinner, H. Suhartono, H. von Briesen (Hoechst AG and Georg-Speyer-Haus, Frankfurt and Medizinische Universitatsklinik Freiburg, Freiburg, all FRG).

Due to the likely role of monocytes/macrophages in the pathogenesis of AIDS there is an urgent need for suitable in vitro systems to study the behaviour of HIV infected monocytes/macrophage cultures. Human blood-born monocytes/macrophages were cultivated on hydrophobic membranes as described elsewhere in the presence or in the absence of lymphocytes (R. Andreesen, J. Immunol. Methods, 56: 295-304 (1983)). Cells were infected in tubes with hydrophobic surfaces and were plated out into 24 well plates. Reverse Transcriptase activities in supernatants of these cultures range from 5 000 to over 2 million cpm/ml. Virus production in infected cultures of monocytes/macrophages could be demonstrated by Reverse Transcriptase-assays for many weeks. We present Reverse Transcriptase data from cultures of HIV infected monocytes/macrophages using different HIV strains, different maturation states of monocytes/macrophages; cytokines were tested also in these cultures. Our system allows to characterize HIV infected monocytes/macrophages as well as different strains of virus.

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ADMINISTRATION OF HUMAN RECOMBINANT INTERLEUKIN 2 ENHANCES ANTI LISTERIA RESISTANCE. Mary Haak Frendscho and Charles J. Czuprynski, School of Vet. Med., Univ. Wisc., Madison, WI 53706.

Interleukin 2 is an important immunoregulatory peptide for T cell activation and proliferation. Resistance to Listeria monocytogenes is known to be T cell dependent, therefore, we examined the effects of administration of human recombinant interleukin 2 (hrIL 2) on resistance to listeria infection. We report here that intravenous injection of hrIL 2 significantly enhanced antibacterial resistance in both BDF1 and C3H/HeJ mice. The effect of hrIL 2 was dose dependent; limited protection was observed with 100 units per mouse whereas optimum protection occurred at 1000 units per mouse. HrIL 2 was equally protective when administered concominant with the listeriae or up to 24 hours prior to infection; it had little effect if given after the bacterial challenge. Kinetic experiments indicated that both the peak bacterial burden and the time lag before L. monocytogenes began to be cleared from the spleen and liver were reduced in hrIL 2 treated mice as compared to control mice. Histopathological examination of spleens and livers confirmed that hrIL 2 treated listeria infected mice experienced considerably less damage to these organs than did control mice. In addition, hrIL 2 treatment significantly enhanced the DTH response of listeria infected mice as compared to control mice. Listeria infected mice exhibited depressed levels of spleen cell mitogenesis. HrIL 2 treatment of infected mice resulted in near normal levels of splenocyte proliferative responses. Administration of hrIL 2 alone had no effect on spleen cell proliferation in response to listeria antigens or to mitogens. These data indicate that hrIL 2 may be an important mediator in host defense against the facultative intracellular pathogen Listeria monocytogenes. Furthermore, they suggest that hrIL 2 might prove beneficial in prophylactic immunotherapy of bacterial infections.

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INTERLEUKIN-1-ALPHA ENHANCES PHAGOCYTOSIS AND KILLING OF M. TUBERCULOSIS AND M. AVIUM COMPLEX BY HUMAN MACROPHAGES. K. Sankaran, R. Swartz, and H. Yeager, Jr. Georgetown Univ. Med. Center, Washington, D.C. 20007

Since monocytes (PBM) from tuberculosis (Mtb) patients produce increased amounts of IL-1, we studied whether IL-1 has a direct effect on PBM phagocytosis and killing of mycobacteria. PBM enriched cultures were incubated on coverslips for 18 hr with or without autologous serum (AHS) in 24 well plates. The supernatant was removed and the cultures washed with warm Hanks' Balanced Salt Solution (HBSS). Equal numbers of bacteria were added to the PBM and incubated with or without IL-1 at 37°C, 10% CO_2. After 2 hr, coverslips were removed, washed in HBSS, fixed in methanol, stained for acid fast bacilli (AFB), and examined microscopically for the percentage of PBM containing $\geq$ 1 AFB. The effect of IL-1 on killing mycobacteria was studied by adding various concentrations of IL-1 to the cultures after ingestion of mycobacteria. At various times, the supernatants and cell lysates were collected, incubated on M7H10 agar, and the M. avium complex (MAC) colony forming units counted after 14 days, Mtb after 8 weeks. IL-1 alone enhanced phagocytosis of Mtb (50-100 U/ml), but was more effective in the presence of serum ($\geq$ 10 U/ml). IL-1 had no significant effect on the phagocytosis of MAC in the absence of serum, but when serum was present, IL-1 at 50-100 U/ml enhanced MAC phagocytosis . Preliminary results suggest that 25-50 units of IL-1/ml enhances the mycobactericidal activity against both Mtb and MAC by nearly 50% more than that of control PBM at 6 days. In summary, IL-1 in addition to its other known effects may have a role in directly enhancing macrophage handling of mycobacteria.

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EFFECT OF GLUCOCORTICOIDS ON MACROPHAGE INSTRINSIC RESISTANCE TO HERPES SIMPLEX VIRUS. C.E. Milligan and W. L. Dempsey. Med. College of PA, Philadelphia, PA 19129

Glucocorticoids released during a stress response have been demonstrated to exert a number of effects on lymphocytes and macrophages (MO). Because stress often alters host resistance, the effects of glucocorticoids on MO intrinsic resistance to herpes simplex virus type I (HSV-I) was determined. A serum-free, chemically defined culture system was chosen for these assays in order to reduce the effects of hormones contained in fetal bovine serum in the assays. Three serum-free chemically defined media were compared for their ability to support short term (up to 72 hours) cultures of resident peritoneal MO from CD-1 outbred female mice. All three media tested [PC-1, HL-1 (Ventrex Labs, Inc.), and HB102 (Hana Media, Inc.) maintained resident peritoneal MO in culture. HL-1 was chosen for the experiments because of lower protein content of the media, and slightly better maintenance of cell number and ectoenzyme profile of the cultured resident peritoneal MO. MO were continuously co-cultured with various concentrations of glucocorticoids. Neither dexamethasone nor methylprednisolone (10^{-3} - 10^{-9}M concentrations) affected survival in culture or ectoenzyme phenotype of the resident peritoneal MO. MO intrinsic resistance to HSV-I as measured by recovery of infectious virus from 2-72 hours post infection was also unaffected by any concentration of dexamethasone or methylprednisolone tested. Hydrocortisone (10^{-3} - 10^{-9}M) had no effect on MO survival or ectoenzyme phenotype. In addition, hydrocortisone (10^{-5} - 10^{-9}M) had no effect on the expression of HSV-I early antigen ICP4 in infected MO. In one experiment, however, MO cultured with 10^{-8} M and 10^{-9}M hydrocortisone exhibited reduced intrinsic resistance to HSV-1. Confirmatory experiments for reduced intrinsic resistance to HSV-I in MO cultured with hydrocortisone are in progress. (Supported by Office of Naval Research N 00014-87-K-0386)

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ACQUIRED IMMUNITY TO M. BOVIS AND M. INTRACELLULARE IS INFLUENCED BY THE **Bcg** GENE. E. Buschman, Y. Goto* and E. Skamene. Montreal General Hospital Research Institute, Montreal, Canada H3G 1A4 and *Department of Cellular Immunology, National Institute of Health, Tokyo, Japan.

The early growth of Mycobacterium bovis BCG and M. intracellulare (Mino) in the mouse is controlled by the **Bcg** gene which exists in two allelic forms, **Bcg^r** (resistant) and **Bcg^s** (susceptible). In the unimmunized mouse, the **Bcg** gene regulates macrophage priming for activation. In this study, we have asked whether cellular immunity arising after infection is also influenced by the **Bcg** gene. The immune response was studied by analyzing in vitro antigen specific lymphocyte proliferation in **Bcg** congenic strains on the BALB/c background; the **Bcg^r** C.D2 and the **Bcg^s** BALB/c. Following intravenous infection with either BCG or Mino, striking similarities were observed in the purified protein derivative (PPD) specific responses. There was a strong T cell proliferative response at 3 weeks after infection in the resistant **Bcg^r** C.D2 mice which was attributed to the Lyt 1.2+ T helper subset. No T helper cell proliferation was detected in the susceptible **Bcg^s** BALB/c mice within the first three weeks of infection, although the B cell response was significant. Furthermore, cell mixing studies showed that spleens of Mino-infected BALB/c mice contained T suppressor cell activity. The results suggest that the **Bcg** gene influenced the regulation of immune responses. The mechanism is unknown at present but it is possible that **Bcg** - regulated macrophage function (e.g. antigen presentation) may affect the activation of T cells. It is concluded that the **Bcg** gene not only regulates natural resistance but also governs the subsequently induced acquired cellular immune responses.

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SUSCEPTIBILITY TO A MURINE RETROVIRUS-INDUCED IMMUNODEFICIENCY SYNDROME IS CONTROLLED BY THE H-2 COMPLEX. D.H. Bourassa, F. Gervais and E. Skamene. McGill Centre for Host Resistance, Montreal General Hospital Research Institute, Montreal, Quebec, Canada H3G 1A4.

LP-BM5 MuLV, a mixture of ecotropic and murine mink cell focus forming type C retrovirus, when injected into susceptible mice, induces a disease which resembles AIDS (MAIDS: murine AIDS). Susceptibility to the disease varies among inbred mouse strains: C57BL/6J (B) mice are susceptible while A/J (A) mice are resistant. Profound impairment of the splenocyte lymphoproliferative response to mitogens is seen at 6 weeks after the infection of susceptible B mice and they succumb shortly thereafter. By 3 months post-infection, B mice exhibit gross lymphadenopathy, splenomegaly and enlargement of the thymus. These changes do not occur in LP-BM5-infected resistant A mice. Genetic control of resistance/susceptibility to MAIDS was determined using a set of 25 recombinant inbred (RI) mouse strains derived from susceptible B and resistant A progenitors (AXB/BXA). Typing of resistance/susceptibility to MAIDS was based on two criteria: the magnitude of splenocyte mitogenic response at 6 weeks after infection and the gross pathologic evaluation of lymphoid organs at 14 weeks of infection. RI strains segregated into two distinct groups. Susceptible mice showed a profound decrease in their lymphoproliferative response to mitogens and a gross enlargement of lymphoid organs. Resistant strains did not exhibit any of these marked changes. When the strain distribution pattern (SDP) of resistance/susceptibility to MAIDS was compared with that of 153 other allelic markers, a strong correlation between the susceptibility and the inheritance of H-2b haplotype was found. On the other hand, the inheritance of H-2a allele made the hosts genetically resistant to the disease. We conclude that genes within H-2 complex are the major regulators of susceptibility to MAIDS. We thank Dr. Herbert C. Morse III (NIAID-NIH) for supplying us with the LP-BM5 MuLV preparation.

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Isolation of Adherence Specific cDNA Clones from a Monocyte cDNA Library. S. Sporn, M. Ladner, G. Martin and S. Haskill, Depts. of Micro. and Immunol., Ob/Gyn and Lineberger CRC, Univ. of North Carolina, Chapel Hill, N.C. 27599. Cetus Corporation, Emeryville, CA 94608.

Adherence to endothelium or extracellular matrices is likely to play a critical role in triggering monocyte activation in extravascular sites of infection, chronic inflammatory disorders, tissue damage and neoplastic growth. We have shown that within 30 minutes of monocyte adherence there is initiation of a complex set of regulatory events as defined by rapid changes in mRNA levels of several genes. In order to analyze these molecular events that are involved in the regulation of induction of gene expression by adherence, we have constructed a cDNA library from monocytes adhered for 30 minutes to plastic in the presence of serum. Following differential hybridization with cDNA probes directed toward adhered and non-adhered monocyte RNA, 35 clones preferentially expressed in adhered monocytes were isolated. Cross-hybridization experiments identified 13 clones, induced greater than ten-fold, which correspond to unique mRNAs. We have done initial characterization of three clones, C2, C6 and C9. Partial sequence analysis indicates that these are unique clones with no homology to known sequences. All three clones are super-induced in monocytes treated with cycloheximide. In addition, these clones demonstrate differential regulation under various conditions including stimulation by LPS, PMA, FMLP, and calcium ionophore. They also exhibit separate induction patterns in CMV infected and PMA treated ML3 cells and in monocyte adherence to endothelial cells.

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BIOCHEMICAL BASIS OF CONTINUOUS I-A EXPRESSION BY MACROPHAGES FROM MICE RESISTANT TO *MYCOBACTERIUM BOVIS* (STRAIN BCG). L. Vespa, J. Nath, W. Lafuse and B.S. Zwilling (Spon: B.S. Zwilling). The Ohio State University, Columbus, OH 43210.

The expression of class II major histocompatibility complex (MHC) (Ia) glycoproteins by mononuclear phagocytes is important for T cell recognition of antigen. We have previously shown that macrophages from strains of mice that are resistant to BCG will continuously express Ia while macrophages from BCG susceptible mice transiently express Ia. We linked continuous Ia expression to BCG resistance by showing that macrophages from Bcg^s BALB/c mice transiently express Ia while macrophages from the BALB/c congenic C.D2Bcg^r mice continuously expressed Ia following production with high concentrations of interferon (IFN)-gamma. The purpose of this investigation was to explore the biochemical basis of continuous Ia expression. Using macrophages from both BALB/c and C.D2Bcg^r mice, which differ only in a 30 centimorgan segment of chromosome 1 derived from Bcg^r DBA.2 mice, we found that macrophages from both strains of mice stopped synthesizing class II glycoprotein within 4 days of in vitro culture. Despite the lack of synthesis, 80% of the macrophages from C.D2Bcg^r mice continued to express Ia compared to only 22% of the macrophages from Bcg^s BALB/c mice. The induction of transient Ia expression by macrophages from both Bcg^r and Bcg^s mice was accompanied by the degradation of the glycoprotein. In contrast, the induction of continuous expression of Ia by macrophages from Bcg^r mice with high levels of rIFN-gamma prevented degradation. The degradation of the class II glycoprotein by macrophages that transiently express Ia could be prevented by the addition of chloroquine. In the presence of chloroquine, 48% of the macrophages from the Bcg^s mice expressed Ia while only 25% of the macrophages expressed Ia in the absence of chloroquine. These studies suggest that one effect of the Bcg gene is to prevent the degradation of Ia. Supported by AI 22249.

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THE NON-HYDROLYSABLE GUANOSINE-5'-TRIPHOSPHATE ANALOG, GUANOSINE-5'-O-(3-THIOTRIPHOSPHATE) (GTPgammaS), ACTIVATES PHOSPHOLIPASE C-MEDIATED HYDROLYSIS OF INOSITOL PHOSPHOLIPIDS IN MURINE HELPER T CELL CLONES. E. Bonvini, K. E. DeBell, T. Hoffman, R. J. Hodes, and M. S. Taplitz. Lab. Cell Biology, DBBP, CBER, US-FDA, Bethesda, MD 20892, and Experimental Immunology Branch, DCBD, NCI, NIH, Bethesda, MD 20892.

Several pieces of evidence have suggested that perturbation of the TcR complex is associated with the rapid hydrolysis of inositol phospholipids (InsPL) by phospholipase C (PLC), leading to the production of diacylglycerol (DAG) and inositol phosphates (IP's). DAG and certain IP isomers (i.e. $I(1,4,5)P_3$ or $I(1,3,4,5)P_4$) may act as "second messengers" by activating protein kinase C (PKC) or mobilizing Ca^{2+}. To gain information on the regulation of PLC activation and its coupling to the TcR, we have studied the role of GTP and its non-hydrolysable analog, GTPgammaS, on the induction of InsPL breakdown by murine, antigen--specific, T "helper" clones. Since guanylyl nucleotides do not cross the plasma membrane, cells were permeabilized by treatment with streptolysin O (SLO). Exposure of SLO-treated cells to GTPgammaS resulted in the rapid generation of IP's. GTP was ineffective, presumably due to its hydrolysis. Neither nucleotide had any effect if SLO treatment was omitted. The effect of GTPgammaS was increased by Ca^{2+} in the pCa range 5-8; higher concentrations of Ca^{2+} decreased or abrogated the response. Exposure of SLO-treated cells to Ca^{2+} in the pCa range 3-8 did not induce InsPL hydrolysis. These results suggest that PLC activity of murine T lymphocytes may be regulated via a GTP-binding protein.

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MONOCYTE(M)-TROPIC AND T LYMPHOCYTE(T)-TROPIC STRAINS OF HIV; REPLICATION IN CULTURED HUMAN MONOCYTES

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The monocyte is an important target for human immunodeficiency virus (HIV), and we are studying the variables which determine HIV-monocyte interaction. We have compared the replication of HIV strains AD and DV, putative monocyte-tropic isolates, with 3B, an established T lymphocyte tropic strain. We utilized lymphocyte-enriched monocyte-depleted nonadherent peripheral blood mononuclear cells, the T lymphocytic cell line SUP-T1, the monocytoid cell line U937, monocyte-enriched lymphocyte-depleted adherent peripheral blood mononuclear cells, and stringently purified adherent monocytes. In monocyte-enriched or stringently purified monocytes, AD replicates to moderate levels, DV replicates to low levels, and 3B fails to replicate and cannot be readily rescued by cocultivation. The monocytoid U937 cell line is very permissive for both 3B and DV; both viruses replicate more efficiently in U937 clones which are high CD4 expressors than in clones which are low CD4 expressors. 3B and DV replicate in nonadherent peripheral mononuclear cells while AD fails to replicate to significant levels; both 3B and DV replicate in SUP-T1 lymphoid cells. In summary, AD virus is a monocyte(M)- tropic virus which replicates in adherent monocytes better than in lymphocyte-enriched mononuclear cultures; DV is an amphitropic virus which can replicate in T cells as well as to modest levels in monocytes; 3B is a T cell(T)-tropic virus which replicates very well in CD4+ cells but not in monocytes. These observations support the hypothesis that HIV strains exhibit a spectrum of cell tropism ranging from T-tropic to amphitropic to M-tropic.

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NEUTROPHIL CHEMOTACTIC ACTIVATION DURING EXPERIMENTAL MYOCARDIAL ISCHEMIA AND REPERFUSION. W.J. Dreyer, C.W. Smith, B.J. Hughes, L.H. Michael, R.D. Rossen, M.L. Entman, and D.C. Anderson. Baylor College of Medicine, Houston, TX 77054.

The infiltration of neutrophils (PMNL) has been implicated as a mechanism of injury in ischemia/reperfusion (I/R). Previous studies suggest that proteins of mitochondrial origin released into canine cardiac lymph bind C1q and may activate complement. Therefore, in a canine model of I/R, we examined cardiac lymph (CL) for evidence of chemotactic activity, and PMNL isolated from CL for evidence of activation. Healthy mongrel dogs had surgical placement of a balloon cuff occluder and a doppler flow probe (DFP) about the circumflex coronary artery. Cannulas were placed in the CL duct and in the right atrium. Forty-eight hrs after surgery, venous blood was drawn for isolation of PMNL and pre-ischemic CL samples were obtained. Occlusion, documented by the absence of blood flow past the DFP, was initiated and maintained for 60 min. During occlusion and reperfusion, CL was collected at 30 min intervals up to 6 hrs. PMNL isolated from blood were exposed to CL and examined for shape change (SC) indicative of chemotactic activation and for increased adherence to canine endothelial monolayers. PMNL incubated with CL samples as well as PMNL isolated from CL samples were stained with fluorescein tagged monoclonal antibodies recognizing CD11b (LM2.1) and CD18 (TS1/18) subunits of the CD18 glycoprotein complex. Binding was quantitated by flow cytometry. In 5 dogs with documented myocardial infarction (MI) SC activity markedly increased in CL during the first 30 mins of reperfusion, peaked at 1-2 hrs and diminished by 4 hrs. In contrast, in 3 dogs with no MI no SC activity was demonstrated. In the MI group, CD11b and CD18 expression on both PMNL incubated with CL and PMNL obtained from CL increased significantly over pre-ischemic values during reperfusion up to 3 hrs ($p<0.05$). In 3 dogs with MI, incubation of blood PMNL with reperfusion CL (at peak chemotactic activity) increased PMNL adherence to canine endothelium over pre-ischemic CL samples from 33 ± 10% to 55 ± 6% ($p<0.05$). Thus, cardiac lymph demonstrates evidence of substantial PMNL activation and enhanced PMNL-endothelial adherence following cardiac I/R. We speculate that monoclonal antibodies directed against adherence determinants may prove useful in limiting neutrophil-mediated reperfusion injury.

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ANALYSIS OF IL-1 AND TNF GENE EXPRESSION IN HUMAN SYNOVIOCYTES AND MONOCYTES BY IN SITU HYBRIDIZATION. K.L. MacNaul, J. Parsons, N. Hutchinson, E. K. Bayne and M. J. Tocci. (Spon: P. Davies). Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065, USA.

The expression of IL-1α, IL-1β and TNF-α mRNAs were examined in cultured human blood monocytes and primary human synoviocytes by in situ and Northern blot hybridization. Monocytes from healthy donors and synoviocytes from patients with rheumatoid (RA) or osteoarthritis (OA) were cultured in media containing LPS (1ug/ml) or PBS for 2 to 72 hrs. The mRNAs for each cytokine were detected using in vitro ^{32}P- or ^{35}S- labeled RNA probes transcribed from cloned human cDNAs with SP6 or T7 RNA polymerases. Each experiment was internally controlled for specificity by hybridization with sense and antisense RNA probes. Examination of LPS- or vehicle-stimulated synoviocytes by Northern blot analyses indicated that IL-1β mRNA was the most abundant transcript, followed by TNF-α. IL-1α was the least abundant mRNA. The expression of IL-1β mRNA reached maximum levels at 8 hrs, while TNF-α peaked at 4 hrs. In situ hybridizations performed on synoviocytes showed that ~3% of the total cells from the OA and 2.5-4% of the cells from RA patients expressed high levels of IL-1β mRNA. The number of IL-1β positive cells was ~6X that of unstimulated cultures. IL-1β mRNA was detected only in cells morphologically resembling tissue macrophages. TNF-α and IL-1α mRNAs were not detected in synoviocytes. However, mRNAs for IL-1α and TNF-α were detected in monocytes and in CHO cells expressing rTNF-α, respectively. In LPS-stimulated monocytes, Northern blot analyses revealed that IL-1β was the predominant mRNA, reaching highest levels at 4-8hr. IL-1α mRNA reached maximum levels at the same time but was present at 10-20X lower levels. In situ hybridizations

showed that ~90% of the monocytes contained high levels of IL-1β mRNA compared to unstimulated cells. IL-1α was detected in a high percentage of cells, while TNF-α mRNA was found in only 9% of the cells. These data indicate that a single monocyte is capable of making both IL-1α and β, and that IL-1β is the predominant cytokine expressed in LPS-stimulated monocytes and synoviocytes.

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SHARED 'EARLY RESPONSE' GENE EXPRESSION IN LPS-STIMULATED MACROPHAGES AND PDGF-STIMULATED FIBROBLASTS. T.A. Hamilton, C.S. Tannenbaum, and Y. Ohmori, Cleveland Clinic Foundation, Cleveland, OH 44195.

Differential screening of a cDNA library constructed using RNA from LPS treated murine peritoneal macrophages led to the identification and isolation of recombinant cDNA clones encoding inducible macrophage gene products ranging in size from 1.5 to 6.5 kb. Northern analysis of macrophage RNA showed that four of the genes (D3, D5, D8, C7) were transiently expressed following stimulation of macrophages with LPS and exhibited half-lives of only 2-4 hrs. Furthermore all these genes could be induced in the absence of continuing protein synthesis. Three of four genes (D3, D8, C7) were also induced in macrophages treated either with IFN gamma, IFN beta, or both. When the plasmid cDNAs were used to probe specific mRNA levels in PDGF-stimulated Balb/c 3T3 fibroblasts, expression of two genes (D3 and D8) was observed in a dose and time dependent fashion reminiscent of expression of other previously defined competence or 'early response' genes (eg., c-fos, c-myc, JE, KC). Previous work has clearly documented the similar expression of such fibroblast-derived competence genes in LPS-stimulated macrophages. Thus these two genes appear to be members of a group whose expression is shared among cells stimulated with diverse signals directing the acquisition cellular competence for diverse functions. The rapid and transient nature with which such genes appear implies that at least some of these genes may be important in regulating the activation process itself. The commonality of their expression indicates that they may perform functions of general significance in such developmental processes. Supported in part by USPHS grant CA39621.

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MACROPHAGE Iα ANTIGEN EXPRESSION INDUCED BY IFNγ AND IL-4. H. Cao, R. M. Crawford, R. G. Wolff, C. A. Nacy and M. S. Meltzer, Walter Reed Army Inst. Res., Washington, DC 20307-5100.

Expression of class II antigens on macrophages, necessary for their function as antigen presenting cells during the induction of an immune response, is not constitutive, but carefully regulated by various secreted products. Two T cell-derived lymphokines, interferonγ (IFNγ) and interleukin-4 (IL-4), induce Ia antigen expression on murine macrophages. We compared IFNγ and IL-4 as macrophage Ia antigen inducing factors and found several fundamental differences in their effects. On a molar basis, IFNγ and IL-4 are equipotent in ability to augment macrophage Ia expression: both induce maximal levels of Ia antigen 48 hr after treatment. By ELISA, the maximal level induced by IL-4 is 80% that induced by IFNγ. However, by alkaline phosphatase immunocytochemistry and microscopic analysis, the frequency of Ia positive cells in IL-4 treated cultures is much lower than predicted by ELISA (80% for IFNγ vs 30% for IL-4; 2% for medium alone). Time course analysis showed that the IL-4-induced Ia expression was short-lived and decreased to background by 4 days of culture. IFNγ-induced Ia remained at maximal levels through 6 days of culture.These results were confirmed by Northern blot analysis of Iaα-specific macrophage mRNA. Decrease in Ia expression with time in IL-4 treated macrophages was not due to cell death, lymphokine depletion, or inhibitory factors in culture fluids. Thus, IFNγ and IL-4 may act through different mechanisms for induction of Ia.

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EXPRESSION OF c-*MYC*, c-*FOS* AND c-*FMS* FOLLOWING ENDOTOXIN STIMULATION OF CSF-1 INDUCED MACROPHAGE (MPH) DIFFERENTIATION. N. Ghildyal, M.J. Myers, J. K. Pullen and L. B. Schook, Lab. of Molecular Immunology, Dept. of Animal Sciences, Univ. of Illinois, Urbana, IL 61820.

The expression of c-*fos* and c-*myc* have been associated with the activation of peripheral MPH. This study was performed to determine the relationship of these proto-oncogenes and c-*fms* with the activation of MPH during *in vitro* differentiation. Bone marrow was cultured in the presence of CSF-1 and adherent bone marrow derived MPH (BMDM) were isolated at 3, 5, 7 and 9 d of culture. BMDM were treated with medium or endotoxin for 15, 30, 60, 120, 180 and 1440 min, harvested and total cytoplasmic RNA and nuclear pellets were isolated. In medium treated BMDM, a steady state level of cytoplasmic RNA was observed for all three proto-oncogenes at 3, 5, 7 and 9 d of culture. During differentiation, c-*myc* mRNA levels were highest at 3 d of culture with lower levels observed at latter periods of differentiation. This activity correlated with levels of CSF-1 induced proliferation. In contrast, c-*fms* cytoplasmic RNA levels were low at 3 d of culture and increased in levels till 7 d of culture with lower levels observed at 9 d of culture. Expression of c-*fos* did not change during the differentiation of the BMDM. Nuclear run-off assays revealed that the steady state RNA levels for these proto-oncogenes was reflective of their transcriptional activity. Further analysis of BMDM, demonstrated that c-*fos* and c-*myc* induction peaked at 60 min following endotoxin stimulation during all periods of differentiation; however, the expression of c-*fms* varied during differentiation. Endotoxin treatment of BMDM obtained after 3 and 5 d of culture did not affect c-*fms* levels of RNA whereas treatment of cells at 7 d resulted in the down-regulation of c-*fms* within 60 min of stimulation. Thus, these results suggest that c-*myc* is associated with the proliferative nature of the differentiating MPH and the response of c-*myc* to endotoxin stimulation is similar at all stages of differentiation. The expression of c-*fos* is related to endotoxin induced activation and is independent of the proliferative state of the cell. Regulation of c-*fms* was associated with activation at discrete stages of MPH differentiation. Presently, we are evaluating the regulation of these genes in during interferon-γ stimulation and the association of these genes with antigen presentation and tumoricidal activity. (Supported in part by NIH grant ES-04348).

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PROTOONCOGENE EXPRESSION IN TUMOR-ASSOCIATED MACROPHAGES (TAM): A PARACRINE CIRCUIT IN THE REGULATION OF THE PROLIFERATION OF TAM IN MURINE SARCOMAS. A. Mantovani, E. Erba, F. Fazioli, A. Rambaldi, B. Bottazzi. Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milan, Italy.

The mechanisms involved in the mainteinance of constant levels of macrophages in growing neoplastic tissues are probably complex and involve various factors. Monocytes are recruited from the blood compartment in tumors and tumor-derived chemoattractants have been identified. Tumor associated macrophages (TAM) have been reported to have increased proliferative activity and in situ proliferation may contribute to the macrophage content of tumor tissues. This previous finding was confirmed in the present study in which TAM isolated from 2 murine sarcomas, mFS6 and MN/MCA1, had high levels of proliferative activity (7-11% of cells in S

phase) compared to peritoneal macrophages (1-2% of cells in S phase) as detected by flow cytometry or ^{3}H-thymidine incorporation. In an effort to elucidate the mechanisms responsible for the proliferative activity of TAM, expression of c-fms and M-CSF was investigated in TAM and sarcoma cells. TAM had high levels of mRNA transcripts of the c-fos and c-fms protooncogene, the latter encoding a tyrosine kinase probably identical to the M-CSF receptor, but did not express appreciable M-CSF transcripts. Sarcoma cells had high levels of M-CSF mRNA and released M-CSF activity. These findings outline a paracrine circuit in the regulation of TAM proliferation, involving M-CSF, secreted by sarcoma cells and acting on c-fms expressing TAM. Since TAM from these murine sarcomas have tumor growth promoting activity, a "ping pong" reciprocal feeding interaction may occur between macrophages and neoplastic cells in these tumors.

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TUMOR-INDUCED IMMUNOSUPPRESSION: INHIBITION OF INTERLEUKIN 2 PRODUCTION BY TUMOR CELL PRODUCTS AND A p15E-RELATED PEPTIDE. David S. Nelson, Peggy Nelson, George J. Cianciolo and Ralph Snyderman. Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards NSW 2065, Australia, and Genentech, Inc., South San Francisco, CA 94080.

Tumor cells of all types and species tested have been found to produce, in culture, substances that depress the expression of cell-mediated immunity (delayed-type hypersensitivity reactions in mouse feet). The factors responsible appear to be related immunologically to the retroviral envelope protein p15E. We have measured the effects of tumor products and conjugates of a synthetic p15E-related peptide, CKS-17, on IL2 production by cultured, Con A-stimulated EL4 cells; in this system IL2 production is independent of IL1. The IL2 produced was assayed on a mouse CTLL cell line. Supernatants of cultures of tumors of mouse, human and guinea pig origin inhibited IL2 production in a dose-dependent fashion. CKS-17 conjugates, but not control conjugates, also inhibited IL2 production. Responses to IL2 (with the particular CTLL line used) were less inhibited by tumor products and very slighly inhibited by CKS-17 conjugates, compared with IL2 production. IL2 receptor density, assayed by flow cytometry, was inhibited only in restricted conditions. IL2 production was inhibited whether the tumor products or CKS-17 conjugates were added early or late in the course of culture of stimulated EL4 cells. Inhibition by CKS-17 conjugates was selective in that IL2 production was inhibited to a greater degree than general protein synthesis in EL4 cells, and general protein synthesis by fibroblasts was unaffected. Fractionation of 6 different tumor supernatants on Sephacryl S-300 revealed a single peak of activity of apparent MW~15,000. Inhibition of IL2 production provides a strategically effective means of subversion of host defences by tumors.

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SYNERGISTIC EFFECTS OF CYTOKINES ON HL-60 DIFFERENTIATION AND FUNCTIONAL ACTIVATION. J.B.Liesch, T.J.Krause, T.M.Oberyszyn, R.S. Greco and F.M.Roberston. UMDNJ/Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

Using the promyelocytic HL-60 cell line, we investigated the effects of gamma interferon (gIFN) and tumor necrosis factor (TNF), alone and in combination (1000U/ml, 24-96 hrs) on parameters associated with terminal differentiation and cellular activation. Morphologically, HL-60 cells treated with gIFN and gIFN+TNF became highly vacuolated, with a decreased nuclear to cytoplasmic ratio. These changes were time dependent, with maximum changes occurring at 72 hrs. When the morphological changes were correlated with cell cycle position, the majority of the cells were found to reside in G_0/G_1 of the cell cycle. Using proliferation of the cloned T helper cell line, D10.G4.1 as a measure of the production of soluble (sIL-1) or membrane bound (mIL-1) Interleukin-1 activity, we found that gIFN alone and in combination with TNF increased both sIL-1 and mIL-1 three fold. TNF had no significant effect on either form of IL-1. Using flow cytometric analysis of antibody binding, we found that gIFN, alone and in combination with TNF, induced a significant amount of HLA-DR expression on the surface of HL-60 cells, while TNF alone induced a profound suppression of HLA-DR expression (Co=16%;gIFN=87%;TNF=10%;gifn+TNF=90%). To correlate the HLA-DR expression with functional activation of HL-60 cells treated with cytokines, we used flow cytometry and the hydrogen peroxide sensitive dye, dichlorofluorescin diacetate (DCF). We found that gIFN, TNF alone and in combination induced significant increases in DCF oxidation.

These observations suggest that the combination of gIFN and TNF act synergistically to induce morphologic and functional alterations in promyelocytic HL-60 leukemia cells that are consistent with functional activation and terminal differentiation.

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DEPRESSION OF POLYMORPHONUCLEAR LEUKOCYTE (PMNL) FUNCTION INDUCED BY INFLUENZA VIRUS HEMAGGLUTININ (HA) AND SIALIC ACID (SA)-BINDING LECTINS. J.S. Abramson, L.F. Cassidy, L.S. Wirkler and D.S. Lyles (Spon: D.A. Bass). Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

Infection of PMNL with influenza virus (IAV) inhibits PMNL metabolic activities. We undertook studies to determine whether the HA of IAV mediates this depression. PMNL were incubated with purified HA for varying time periods, and the oxidative response to souble (phorbol myrsitate acetate) and particulate (opsonized zymosan) secondary stimuli was measured using the luminol-enhanced chemiluminescence assay. The HA, in either liposomes or protein aggregates (rosettes), depressed PMNL chemiluminescence to both secondary stimuli. Depression was observed within 2 min of HA incubation with PMNL and lasted for more than two hours. Additional studies examined if binding of HA to SA-containing receptors was adequate to inhibit chemiluminescence or if fusion of HA to PMNL membranes was required (the membrane fusion activity of HA requires proteolytic cleavage of HA, whereas receptor binding activity does not). There was no difference in the ability of virions with cleaved or uncleaved HA to depress PMNL chemiluminescence, suggesting that fusion is not required for PMNL dysfunction. Further studies examined if other SA-specific binding proteins inhibit PMNL chemiluminescence. SA-specific lectins were incubated with PMNL for varying times and the chemiluminescence response to secondary stimuli was measured. PMNL depression due to lectins was similar to that seen with HA or IAV. Thus, IAV attachment to SA-containing receptors is responsible, at least in part, for IAV-induced PMNL dysfunction. (This work was supported by NIH grants AI-20506 and KO4 AI-00670.)

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INFLUENZA A VIRUS (IAV) ALTERS ACTIN DISTRIBUTION IN POLYMORPHONUCLEAR LEUKOCYTES (PMNL). J.G. Wheeler and J.S. Abramson. (Spon: D.A. Bass). Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

Previous flow cytometry analysis has demonstrated that IAV leads to increases in NBD-Phallacidin (NBD-Ph) fluorescence in PMNL treated with virus compared to controls, but 20 sec following FMLP stimulation both virus and buffer treated cells have equal peak F-actin fluorescence. We propose that alterations in actin assembly may explain IAV-induced alterations in PMNL

lysosome-phagosome fusion and chemotactic responses to formyl-methyl-leucyl-phenylalanine (FMLP). To further test this hypothesis, PMNL were exposed to virus or buffer for 15 min at 37°C and then stimulated with FMLP (10^{-7}-10^{-9}M). At various time points PMNL were fixed and stained with the F-actin probe NBD-Ph. Cells were then analyzed by flow cytometry or by computer enhanced fluorescent microscopy. In the latter technique, individual polarized cells were analyzed to determine local fluorescence intensity in the lamellipodium, uropod and body of the cells. In addition, experiments were carried out to determine the relative amounts of actin recovered in the detergent insouble (1% Triton) cytoskeleton by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Five min and 10 min after FMLP stimulation, virus treated cells had significantly increased F-actin fluorescence ($p<.01$, n=5). Ten min after addition of FMLP, fluorescence microscopy studies showed significant increases in the F-actin polarization within PMNL with greater lamellipodial to uropod fluorescence ratios in virus treated cells ($p<.01$, n=3). SDS-PAGE studies of Triton extracts of PMNL (8,000xg precipitates) showed increases in associated actin from cells incubated with virus both before and 20 sec after FMLP stimulation (n=3) in the presence or absence of calcium and magnesium. These studies show that IAV alters several aspects of actin function in PMNL. Dysfunction of actin polymerization may therefore mediate a number of altered functions in PMNL.

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DIFFERENTIAL EFFECT OF PENTOXIFYLLINE ON RESPONSE OF NEUTROPHILS TO CHEMOTACTIC PEPTIDE (fMLP) AND PHORBOL ESTER (PMA) MS Currie, KMK Rao, J Crawford, and HJ Cohen (Spon: J Brice Weinberg). Duke and DVAMC, Durham, NC 27705

Pentoxifylline (Pf), a xanthine derivative used to treat symptoms of peripheral vascular disease, increases blood cell deformability, and enhances chemotaxis in conditions associated with decreased neutrophil deformability. We have shown that Pf causes a dose-dependent decrease in the amount of polymerized actin (F-actin), and inhibits capping of fluoresceinated Con-A in lymphocytes and neutrophils (Clin Res 36:368a), not accounted for by changes in cyclic nucleotides. We here report the differential effect of Pf on the respiratory burst and degranulation responses of neutrophils induced by fMLP and PMA. F-actin was measured by NBD-phallicidin binding. Superoxide production was measured by rate of cytochrome c reduction, and degranulation was assessed as myeloperoxidase (MPO) and lysozyme activity in supernates of cells stimulated in the prescence of cytochalasin B. Pf produced a dose-dependent inhibition of fMLP-induced but not PMA-induced superoxide generation. Pf inhibited fMLP- and to a lesser extent, PMA-induced degranulation. Degranulation, expressed as log enzyme activity, was inversely proportional to Pf concentration from 0-10 mM.

stimulus:	ΔO_2^-/min	MPO	lysozyme
10^{-7}M fMLP	17 nM	88±2	22±5
" + 10 mM Pf	2 nM	7±2	7±3
10^{-6} M PMA	8 nM	47±16	17±5
" + 10 mM Pf	8 nM	13+ 1	10+4

Pf reduced F-actin content even after pertussis toxin (which did not alter baseline F-actin, but abolished the fMLP-induced increase). Despite reduced baseline F-actin, the dose response to fMLP (10^{-10} to 10^{-6}) measured as % increase in F-actin in the presence of 10 and 1 mM Pf was maintained or enhanced (respectively). These data suggest that Pf has effects in neutrophils independent of effects on protein kinase C, pertussis-toxin sensitive G proteins, and cyclic nucleotides, which may account for reduced deformability and enhanced chemotaxis. The differential effect of Pf on superoxide generation induced by fMLP vs PMA, along with the preservation of fMLP-induced actin polymerization at reduced levels of total F-actin suggest an effect on restricted aspects of chemotactic peptide-receptor mediated signal transduction, possibly related to the activation and/or activity of phospholipase C, its substrates or products.

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INVOLVEMENT OF PROTEIN KINASE C (PKC) IN THE ACTIVATION OF fMET-LEU-PHE (fMLF)-MEDIATED RESPIRATORY BURST IN HUMAN NEUTROPHILS (PMNs). J. Nath and A. Powledge (Spon: C. Nacy). WRAIR, Washington, DC 20307-5100

Based on the differential inhibitory effects of protein kinase inhibitors C-I and H-7 on fMLF and PMA-induced superoxide (O_2^-) generation in PMNs, other investigators have proposed a PKC-independent pathway for the activation of the NADPH oxidase-mediated respiratory burst in fMLF-stimulated PMNs. Contrary to these reports, we observed a marked inhibition (>70%) of fMLF-stimulated O_2^- production by 10^{-6}M C-I or H-7. The observed inhibition was a highly temperature-sensitive event and occurred only when C-I or H-7 was added to PMNs at physiologic temperature, i.e. at 37°C. In carefully monitored studies, when the temperature at which C-I or H-7 added to PMNs was varied between 16°C to 37°C, no significant inhibition of fMLF-stimulated O_2^- production by C-I or H-7 was observed even at 35°C. However, when added at 37°C, both the maximal rate and the final extent of fMLF-stimulated O_2^- production were significantly inhibited by 10^{-6}M C-I or H-7. A relatively weaker PKC antagonist, HA-1004, was not inhibitory under identical experimental conditions. In contrast, the inhibition of PMA-induced O_2^- production by 10^{-6}M C-I or H-7 was not temperature-dependent, and occurred regardless of the temperature at which C-I or H-7 was added to PMNs. These results suggest that either the PMN membrane fluidity (at 37°C) or some other temperature-dependent metabolic event, is critically involved in the observed inhibition of fMLF-induced O_2^- production by C-I or H-7. Furthermore, the present findings argue against the existence of a PKC-independent pathway for the activation of the respiratory burst in fMLF-stimulated PMNs, and are consistent with recent reports of inhibition of fMLF-induced O_2^- production by the PKC inhibitors sphinganine and sphingosine.

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DEGRANULATION AND ACTIVATION OF THE RESPIRATORY BURST IN HUMAN NEUTROPHILS. D.L. Schneider, F.S. Manara and J. Chin Dartmouth Medical School, Hanover, NH 03756.

The relationship between degranulation and activation in resting, primed, and activated neutrophils was investigated to determine whether degranulation could be a mechanism for activation of the respiratory burst. If there is a translocation of intracellular membrane components to the plasma membrane, there should be an increase in membrane at the cell surface. We show that impermeant agents label 3-4 times more membrane components in the activated neutrophil than in the resting cell. These components do not represent more of the same or cryptic plasma membrane components because there is no increase in labeling with an antibody to a plasma membrane protein marker. Rather, results with an antibody to intracellular granule components show that there is a 1.5-2.0 fold increase in granule components at the cell surface. These results suggest that translocation of granule components increase in the activated cells over that in the resting cells. If degranulation is involved in activation, then inhibitors of membrane fusion or secretion should inhibit oxidase activation. We present evidence that increasing the osmolarity of the medium inhibits both degranulation and oxidase activation. Furthermore, we also present evidence that degranulation is temperature-sensitive in a manner parallel to the respiratory burst, and neither degranulation nor activation occur below 17°C. However, if cells are primed, we find that activation below 17° occurs. Thus priming by cytochalasin B and lithium chloride may be due to degranulation or translocation of intracellular membrane to the plasma membrane. An involvement of degranulation in the activation of oxidase activity in human neutrophils is suggested.

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EFFECTS OF RECOMBINANT BOVINE INTERFERON-ALPHA AND INTERFERON-GAMMA ON BOVINE NEUTROPHIL FUNCTIONS. Allen K. Sample and Charles J. Czuprynski. Univ. of Wisconsin-Madison, Wisconsin, WI 53706.

Neutrophils play a critical role as a first line of defense against infectious agents. It has recently been recognized that neutrophil functions can be enhanced both in vivo and in vitro by a number of immunoregulatory cytokines, including interferons. We have examined the in vitro effects of recombinant bovine interferon-alpha (IFN-α) and interferon-gamma (IFN-γ) on the oxidative response and functional activities of bovine neutrophils. Incubation of neutrophils with as much as 10^4 Units per ml of IFN-α for two hours had no effect on luminol-dependent chemiluminescence, superoxide anion generation, nor hydrogen peroxide production following stimulation with opsonized zymosan. In contrast, treatment of neutrophils with as little as 2 Units per ml of IFN-γ enhanced chemiluminescence and hydrogen peroxide production, but not superoxide anion generation, in response to opsonized zymosan. Neither interferon was directly chemotactic for bovine neutrophils, nor did treatment with either IFN-α or IFN-γ affect the directed migration of neutrophils towards zymosan activated serum. The increased oxidative response of IFN-γ treated neutrophils was not the result of enhanced phagocytosis; IFN-γ treatment had no effect on either the percentage of neutrophils with ingested zymosan nor the number of zymosan particles per neutrophil. These results suggest that IFN-γ, but not IFN-α, prime bovine neutrophils for increased oxidative activity in vitro.

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DIFFERENTIAL EFFECTS OF ENDOTOXINS ON THE TERMINAL DIFFERENTIATION AND FUNCTIONAL ACTIVITY OF HUMAN MONOCYTE/MACROPHAGES. R. Andreesen, W. Brugger, D. Waltersbacher, H. Sawert, L. Kanz, A. Rehm, C. Galanos, R. Engelhardt, G.W. Löhr. Medizinische Klinik, Hugstetter Straße 55, D-7800 Freiburg, FRG.

Blood monocytes (mo) undergo maturation into macrophages (MO) upon migration from the capillary bed to tissue sites of inflammation at which they are exposed to environmental signals. Functional competence and phenotype expression is the result of both differentiation inducing and activating events. Monocyte to MO maturation can be followed in vitro by the expression of specific maturation-associated antigens of the MAX series which are measured by the cell-ELISA technique. Here we report that bacterial lipopolysaccharides (LPS) and Lipid A very efficiently inhibited the serum-induced maturation of human mo in vitro. At the same time LPS induced the up-regulation of CD14 antigens and down-regulation of HLA-DQ antigens. Optimal activity was seen in the nanogram range with higher concentrations being less effective. Inhibition of maturation was not due to secondary LPS induced signals like interleukin-1 (IL-1) or tumor-necrosis factor whereas interferon-alpha might be involved. When tested on cells of defined maturation stages LPS induced IL-1 secretion in mo but not in MO but stimulated the release of high amounts of colony-stimulating activity in MO and only to a lesser extent in freshly isolated mo. Simultaneously, MO tumorcytotoxicity and MO secretion of the maturation-associated monokines alpha-2-macroglobulin, transferrin, and fibronectin did not change upon LPS treatment. The results indicate that endotoxins may influence MO biology distinctively and interfere both with the maturation process as well as with the functional activity. Endotoxins may thereby regulate the levels of endogenous pyrogen as well as hematopoietic and immunoregulatory molecules.

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PRODUCTION OF TWO NOVEL NEUTROPHIL CHEMOTACTIC PEPTIDES BY LPS-STIMULATED ENDOTHELIAL CELLS. J.-M. Schröder and E. Christophers, Dept. of Dermatology, Univ. Kiel, 2300 Kiel, FRG.

Intradermal injection of lipopolysaccharides causes a local inflammatory response characterized by neutrophil accumulation in the tissue. Because principally endothelial cells could mediate leukocyte infiltration we asked whether LPS stimulates umbilical vein endothelial cells (HUVEC) to produce neutrophil attractants. Cultured HUVEC were stimulated with LPS (100 ng/ml) for 24 hrs. Thereafter media were collected and separated by G-75 chromatography. Neutrophil attractants as determined by a Boyden chamber chemotaxis assay eluted near 10 kDa. Biological active fractions were further purified by subsequently performed wide pore RP-8-HPLC, TSK-2000-HPLC, narrow pore RP-18-HPLC, and Poly-F-HPLC. As a result two apparently pure polypeptides, termed α- and β-endothelial cell derived neutrophil activating peptide (ENAP), were detected demonstrating a single line upon SDS-PAGE at 15 kDa and 7.5 kDa, respectively. Both factors elicited half maximum chemotactic responses of PMNL at 30 ng/ml, and 2 ng/ml, respectively, whereas release of azurophilic granule β-glucuronidase of cytochalasin B pretreated PMNL occured at nearly 10 fold higher doses. Cross-desensitization-experiments revealed cross-reactivities between both chemotaxins and recently purified monocyte (MONAP) and lymphocyte-derived neutrophil attracting peptides (LYNAP), not however with C5a or FMLP. In addition, the 7.5 kDa factor (β-ENAP) showed chemotactic potency comparable to that of MONAP indicating that ENAP belongs to the same family of recently detected novel neutrophil attracting cytokines.

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ROLE OF 1,25-DIHYDROXYCHOLECALCIFEROL AS AN AUTOCRINE MACROPHAGE REGULATOR. D R Katz, A Brennan, I Ziegler, D S Latchman, M Hewison, J L H O'Riordan. Univ Coll/ Middlesex Sch Med, LONDON W1P 8AA, U.K..

The active metabolite of vitamin D3, 1,25-dihydroxy-cholecalciferol, (1,25DHCC) has been studied extensively as an in vitro inhibitor of proliferation for myelomonocytic cell lines; and at the same time it has also been regarded as a useful tool to facilitate the study of the cellular and molecular biology of differentiation along the pathway which leads towards the peripheral mononuclear phagocyte phenotype. The hormone is itself known to be a macrophage product. Previously we have demonstrated that differentiation is associated with increased expression of the nuclear receptor for the metabolite, and that the receptor has a higher affinity for the active form of the hormone than for the less active dihydroxy- compound, 24, 25DHCC. In recent studies we have examined how these different effects are related to the biosynthetic pathway of the hormone itself, and to the functional activity of the mononuclear phagocytes. Assays for 1-alpha hydroxylase (1-OHase) and 24-alpha hydroxylase (24-OHase) show that in 1,25 DHCC-induced macrophages the synthetic 1-OHase enzyme is not detectable, but the inactivating 24-OHase enzyme is present. Substrate inhibition of synthesis is not seen when phorbol esters are used as differentiating agents: in the phorbol-treated cells the 1-OHase activity is demonstrable in parallel with receptor induction. Radiolabelled antibodies have been used to show that 1,25DHCC-differentiated and phorbol differentiated cells express increased amounts of the CD4surface receptor; and both populations are permissive for viral infection by herpes

simplex virus. These findings add emphasis to the hypothesis that the D3 metabolic pathway is as important in the tissue mononuclear phagocyte microenvironment as it is in the bone marrow. The local DHCC metabolites may influence inflammation and immunity not only be inhibiting responder T cells, as we have shown previously, but also by acting as an autocrine macrophage regulatory mechanism for responsiveness in viral disease processes.

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13-CIS RETINOIC ACID (13cRA) INCREASES MACROPHAGE PRODUCTION IN INTERLEUKIN-3 (IL-3) STIMULATED MOUSE BONE MARROW CULTURES. S.G. Bender, C.C. Stewart, and R.A. Habbersett, Dept. of Pathology, Univ. of New Mexico, Albuquerque, NM, and LANL, Los Alamos, NM.

Bone marrow cells in liquid culture with IL-3 generate non-adherent granulocytes, mast cells and macrophages. 13cRA was added with IL-3 to bone marrow cultures. Growth kinetics were followed by quantitating adherent cells using an Artek video counter and non-adherent cells with a Coulter counter. Cell lineages present were identified by flow cytometry using the following antibodies: MAC1 (granulocytes and macrophages), F4/80 (macrophages), FE4.2 (mast cells) and HI2 (Thy1 + myeloid precursors). Colony forming cells (CFC) were assessed in agar cultures stimulated with IL-3, GM-CSF, or M-CSF. 13cRA (10^{-8}-10^{-6}M) enhanced proliferation of the non-adherent cells and concentrations > 5 x 10^{-7}M stimulated a 6X increase in adherent macrophages. Four color flow cytometric analysis indicated a 2X increase in the MAC1 + F4/80+ cells which were sorted and identified morphologically as macrophages. 13cRA also increased by 60-95% the numbers of CFC responsive to IL-3 and M-CSF but decreased by 40% the CFCs responsive to GM-CSF. This suggests that 13cRA increases the production of macrophages by modulating the commitment of IL-3 expanded progenitor cells to the macrophage lineage.

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MODULATION OF TUMOR NECROSIS FACTOR (TNF) RELEASE BY RETINOIDS IN MURINE PERITONEAL MACROPHAGES.
Kapil Mehta, Department of Clinical Immunology and Biological Therapy, UT M.D. Anderson Cancer Center, Houston, TX 77030.

Serum retinoids are potent inhibitors of gamma interferon-lipopolysaccharide induced activation of mouse macrophages. This retinoid-induced suppression of macrophage activation is associated with induction of a protein cross-linking enzyme, tissue transglutaminase (TGase). Our recent studies demonstrate that TNF-α can serve as an endogenous substrate for tissue TGase. Tissue TGase did not induce intra-molecular (TNF-TNF) cross-linking. Thus, ^{125}-I labelled TNF when incubated in presence of purified TGase and Ca^{+2} ions, caused no cross- linking of TNF as revealed by PAGE electophoresis and autoradiography. However, inclusion of the macrophage lysates to reaction mixtures, resulted into an additional radioactive band at 70 Kd position. Further studies on these lines suggested that retinoid-induced expression of tissue TGase in macrophages catalyses the covalent cross-linking of TNF to a 50 Kd membrane associated protein resulting into an inhibition of TNF release in the spent media. This membrane associated TNF was not active in mediating the tumor cell killing function as demonstrated by its inability to kill the actinomycin D treated L929 cells. Intracellular delivery of retinoids to macrophages by encapsulation within lipid vesicles (liposomes) potentiated the inhibitory effect of retinoids on macrophage tumor cell-killing function. Also, the retinol in its free form which was poor or inactive in inhibiting the IFN-LPS triggered activation of macrophages, became highly effective when presented in liposomal form. These results thus strongly suggest that retnoid-mediated inhibition of macrophage activation is due to post-translational changes induced in TNF molecule.
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INTERLEUKIN-4 (IL-4) EFFECTS ON MONOCYTE PGE_2 AND TUMOR NECROSIS FACTOR(TNF) C.Miller,G.Szabo and T.Takayama, Univ. Mass. Worcester, MA 01655

An interferon γ(INFγ) prime generally augments endotoxin stimulated TNF activity by MØ while concomitantly downregulating MØ PGE_2. We analyzed rhuman IL-4 (33 U/ml) as a prime for MØ TNF in combination with muramyl dipeptide (MDP) (20μg/ml) a synthetic momomer related to peptidoglycan. MØ TNF was measured in the L-M bioassay. rIL-4 downregulated MDP stimulated MØ PGE_2 (assessed in ELISA) by 77+6% while 10^{-6}M indomethacin(Indo), a cyclo-oxygenase inhibitor, decreased PGE_2 levels 99+0.8%. In contrast, Indo in combination with MDP augumented MØ TNF activity while IL-4 decreased MØ TNF activity. These finding were even more striking when the MØ subset selected for high density of the p72 receptor for immunoglobulin G ($FcRI^+$ MØ) was examined. This MØ subset is the primary TNF producing MØ subset in response to MDP+INFγ and is selected by rosetting isolated MØ with anti-RH coated human erythrocytes.

	TNF	PGE_2	TNF	PGE_2
$FcRI^+$	4955	4700	2593	1600
MDP	6653	7100	4367	4700
Indo,MDP	9287	100	6478	100
IL-4,MDP	4456	2100	-	-
IL-4,MDP,Indo	-	-	2146	93
INFγMDP	12229	3900	8493	5000

These data suggest that IL-4 regulates MØ function in a manner selectively different than INFγ. The finding that cyclo-oxygenase inhibitors can increase human MØ TNF release has implications for patients receiving cyclo-oxygenase inhibitors to downregulate immunosuppressive MØ PGE_2 levels. rIL-4 downregulates MØ PGE_2 levels, but does not concomitantly upregulate TNF, suggesting a possible use for rIL-4 in treatment of patients in endotoxin shock.

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ALTERATIONS IN GTP-BINDING PROTEIN IN HUMAN NEUTROPHILS BY INFLUENZA VIRUS. Elaine L. Mills, Garry M. Bokoch, Jon S. Abramson, McGill Univ. Montreal PQ H3H 1P3, Res Inst. Scripps Clinic La Jolla CA 92037, Bowman Gary Sch Med, Winston-Salem NC 27103

Influenza virus has been shown to depress end-stage neutrophil (PMNL) function including chemotaxis and superoxide generation. The virus causes phosphorylation of several cellular proteins in unstimulated PMNL and inhibits phosphorylation of multiple proteins from both cytosol and particulate fractions of stimulated PMNL suggesting that virus interfers with PMNL function prior to phosphorylation in the signal transduction sequence. The present study was done to determine whether the activity of GTP-binding protein (G_c) was affected by virus. Since it is not known whether any G_c protein(s) are phosphorylated, we also determined whether G_c protein was phosphorylated in uninfected and infected cells. PMNL were incubated with influenza virus or buffer for 30 min and purified plasma membranes were stimulated with formylmethionyl-leucylphenylalanine (FMLP) and GTP-ase activity measured. Influenza virus suppressed both stimulated and unstimulated GTP-ase activity in purified plasma membranes by $\geq$ 50%. PMNL were also labeled with ^{32}P, incubated with virus or buffer for 30 min, stimulated with FMLP for 30 sec, and detergent solubilized. The proteins were immunoprecipitated followed by SDS gel electrophoresis and autoradiography. Preliminary studies with specific antibody to the β subunit did

not demonstrate protein phosphorylation. Studies in progress are examining phosphorylation of the α subunit. These results suggest that influenza virus may alter the GTP-ase activity of one or more GTP-binding proteins and inhibit protein phosphorylation and subsequent end-stage PMNL function.

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LIPOPOLYSACCHARIDE-INDUCED EXPRESSION OF THE COMPETENCE GENE, KC, IN VASCULAR ENDOTHELIAL CELLS IS MEDIATED THROUGH PROTEIN KINASE C. X. Shen, T.A. Hamilton, and P.E. DiCorleto, Cleveland Clinic Research Institute, Cleveland, OH 44195.

The KC gene is one of a family of cell cycle-dependent competence genes that was originally identified in platelet-derived growth factor stimulated BALB/c-3T3 cells. We have examined the expression of the KC gene in cultured porcine aortic endothelial cells in response to bacterial lipopolysaccharide (LPS) as a first step in defining the molecular events in endothelial cell "activation." LPS markedly stimulated the steady state level of KC mRNA in confluent endothelial cells without increasing the growth fraction of the cultures. Maximum induction of KC occurred in the cells following exposure to 10 ng/ml LPS for two hours. A series of studies was performed to determine whether protein kinase C was involved as an intracellular mediator of this LPS-stimulated response. Endothelial cells treated with phorbol myristate acetate (PMA) and 1-oleoyl-2-acetylglycerol (OAG) exhibited induction of KC gene expression. A maximum response was obtained with 10 nM PMA, the effect decreasing with higher levels of the phorbol ester. The increased expression of KC induced by LPS and PMA was inhibited by the presence of 50 μM 1-(5-isoquinoline-sulfonyl)-2-methylpiperazine (H7), a protein kinase C inhibitor, but not by an H7 analog HA1004 (50 μM), which is a much less effective inhibitor of C kinase, or by the cyclooxygenase inhibitor acetylsalicylic acid (100 μM). No cytotoxicity due to the various inhibitors was observed in the endothelial cell cultures. These results demonstrate that the KC gene may be induced by LPS in vascular endothelial cells in a proliferation-independent process. Secondly, unlike LPS-induced KC expression in macrophages and PDGF-induced KC expression in 3T3 cells, LPS induction of KC in endothelial cells requires the action of protein kinase C. (Supported in part by HL34727, CA39621 and HL01561)

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ROLE OF PKC IN THE CELL SURFACE EXPRESSION AND PHOSPHORYLATION OF DIFFERENTIATION ANTIGENS OF RESTING AND ACTIVATED HUMAN T CELLS. A. Carrera, L. Cardenas, A. Tugores, M. Cebrian, F. Sanchez-Madrid, M. Lopez-Botet and M.O. de Landazuri. Servicio de Inmunologia. Univ. Autonoma. Hospital de la Princesa. c/Diego de Leon, 62- 28006 Madrid.

We have studied the effect that the activation of Protein Kinase C (PKC) has in the cell surface expression of several human T cell leukocyte differentiation antigens. Treatment of resting T cells with phorbol esters (PMA) produced a dose dependent down-regulation and internalization of CD3 and CD4 and upregulation with augmentation in the expression of CD2, CD5 and CD6. We also studied the role of PKC in the induction of cell surface activation antigens. Two cell surface activation antigens-4F2 and AIM (a recently described molecule) seems to be very much dependent on PKC activation since they appear very early (2-5 hours) after PMA treatment and are fully expressed after 18-24 hours. We also studied if phorbol esters could down-regulate the expression of activation antigens already present on T cells. We observed that the addition of PMA to activated T cells down-regulate the expression of the transferrin receptor whereas it upregulates the expression of IL-2R, 4F2 and AIM. Finally, we analyzed the phosphorylation patterns of these cell surface antigens after activation with PMA. Specific phosphorylations were observed in the case of CD3, CD4, CD5, CD6 and AIM. These data indicate that PMA induced phosphorylation of specific cell surface differentiation antigens can be associated either with down-regulation (CD-4) or with upregulation (CD5, CD6, AIM) of the cell surface molecules.

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PROTEIN KINASE C ISOTYPE DISTRIBUTION AND SELECTIVE ISOTYPE TRANSLOCATION WITH Ca^{2+} IN HUMAN NEUTROPHILS AND CYTOPLASTS T. Fujiki, M. W. Rossi, W. A. Phillips, R. B.Johnston Jr. and H. M. Korchak (Spon: L. Kilpatrick-Smith), Univ. of Pennsylvania, Philadelphia PA 19104.

Protein kinase C (PKC;80kDa) has been proposed as a mediator in multiple cell functions, including activation of O_2^- production. Translocation of PKC from cytosol to membranes is thought to be crucial to PKC activation. Recently, several isozymes of PKC have been identified by cloning technology. Polyclonal, antipeptide antibodies that specifically recognized the consensus as well as α, β, and γ isozyme epitopes (Makowske et al,JBC 263:3402), were used to study the distribution of PKC in disrupted human neutrophils and cytoplasts. Intact neutrophils and cytoplasts (vesicles of cytoplasm enclosed by plasmalemma) produced O_2^- in response to stimulants such as FMLP and PMA. PKC was observed in cytosol and membrane fractions of resting intact neutrophils and cytoplasts with antibody to consensus peptide; the immunoprecipitin reaction was qualitatively less in the pellet than in the cytosol fraction. The presence of Ca^{2+} during disruption of cytoplasts resulted in increased immunoreactive 80 kDa species in the membrane fraction and a reduction in the cytosol. PKC activity, with histone as the substrate, corroborated this result. Antibody to α PKC immunoblotted strongly to a 60kDa polypeptide in cytosol and pellet fractions. No apparent differences in the blotting intensity or pattern were observed in the Ca^{2+}-containing and the Ca^{2+}-depleted conditions. Antibody to β PKC immunobloted predominantly to an 80kDa polypeptide in the cytosol. The immunoprecipitin reaction in the cytosol was qualitatively less in the Ca^{2+}-containing samples than in the Ca^{2+}-depleted samples, suggesting translocation of this isotype. γ PKC antibody immunobloted to an 80kDa polypeptide in the cytosol. No difference in the distribution of this isotype was observed between Ca^{2+}-containing and Ca^{2+}-depleted conditions. These results indicate that 1) neutrophils contain α, β and γ PKC isozymes, 2) the isozymes are differentially distributed and 3) the β isozyme is a translocatable species.

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ROLE OF PROTEIN KINASE C IN THE ACTIVATION OF LIVER MACROPHAGES. D.L. Laskin, C.R. Gardner, A.M. Pilaro and J.D. Laskin. Rutgers University and UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Protein kinase C (PKC) has been postulated to be a critical enzyme in initiating the biological activity of a variety of extracellular signals involved in cellular activation. To study the role of PKC in liver macrophage activation we used synthetic diacylglycerols (DAG) and tumor promoters which are known to bind to and activate PKC. We found that treatment of cultured rat liver macrophages with phorbol myristate acetate (TPA), phorbol didecanoate or mezerein produced morphological alterations in the cells characteristic of activated macrophages. Cells became enlarged, highly vacuolated and spread on culture dishes. Each of these analogs was also found to be potent inducers of macrophage chemotaxis. Synthetic DAG including 1-oleoyl-2-acetylglycerol (OAG), 1,2-dioctanoylglycerol (diC8) and 1,2-didecanoylglycerol (diC10) were

also found to induce morphological alterations and chemotaxis in liver macrophages. Furthermore, phorbol diacetate (PDA) a biologically inactive analog that blocks TPA receptor binding inhibited chemotaxis induced by TPA, OAG as well as the complement fragment C5a. Both DAG and tumor promoters also stimulated phagocytosis of sheep red blood cells by the macrophages. In contrast to the tumor promoters, DAG did not stimulate the respiratory burst as measured by flow cytometry and cytochrome C reduction. These data suggest that some, but not all macrophage functions associated with activation are mediated by PKC. Supported by NIH grant GM34310.

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THE ENHANCEMENT OF RECEPTOR-MEDIATED PHAGOCYTOSIS BY AMPHOTERICIN B MONOMETHYL ESTER (AME). S. Sachs, U.J. Pressia, J.L. Mulicy, and C.P. Schaffner Wakeman Institute of Microbiology @ RUTGERS-The State University, New Brunswick, N.J. 08855-0759

Amphotericin B (AMB) and AME, polyene macrolice antifungal antibiotics, possess immunomodulating activities. Recently, they have attracted attention for their antiviral activity against lipid-enveloped viruses. AME, in particular, can protect H-9 cells (human lymphoblastic cell line) from HIV-1 (AIDS virus). Also, AME is at least 10-fold less toxic than AMB for many different cell types. Since polyene-mediated effects presumably are the result of membrane-sterol interactions, we investigated various physical membrane parameters and membrane-associated immunoreceptors. Binding of FITC labeled ligands to PBL and H-9 cells was insensitive to AME and AMB (at 10-100 ug/ml), as was the binding of hemolysin to sheep red blood cells (sRBC) and the hemolytic action of complement. Both polyenes have antiviral activity in this range. Although AMB and AME both bound to the membranes of cells essentially equally, AMB significantly increased the membrane fluidity, whereas AME did not. In experiments with T lymphocytes and FITC-anti-T3 antibodies, AMB blocked both patch and cap formation, whereas AME blocked only capping. This suggests that polyenes can inhibit cellular internalization of lymphocytic membrane ligand-receptor complexes rather than binding of ligand. Treatment of plastic adherent human PBL with AME, but not AMB, caused a dramatic dose-dependent increase in phagocytosis of antibody-sensitized RBC. Pretreatment of the sensitized RBC also resulted in increased phagocytosis, but not as pronounced. AME has the capacity to enhance Fc receptor-mediated phagocytosis at concentrations which are antiviral for HIV-1. Also, after treating normal PBL with AME prior to mitogenic stimulation, we found that production of IL-2 and BCGF was not affected. Similarly, the IL-2 and BCGF receptor-mediated proliferation of dependent cell lines (CTLL-2 and BCL_6 respectively) was insensitive to AME as was the receptor-mediated cytocidal action of Tumor Necrosis Factor. In this concentration range, AME is neither cytotoxic nor does it adversely affect other receptor-mediated immune functions. The detailed mechanism of AME action needs to be elucidated to exploit polyenes as therapeutic/prophylactic drugs.

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DIFFERENTIATION OF MACROPHAGES (MP) AND NEUTROPHILS (PMN) IS ASSOCIATED WITH CHANGES IN CELLULAR PROTEIN PHOSPHORYLATION. A.A. Sirak, F.H. Mermelstein, J.D. Laskin and D.L. Laskin. Rutgers University and UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

Protein kinases are thought to play an important regulatory role in cell growth and differentiation. In the present studies, we measured changes in protein phosphorylation during differentiation of HL-60 promyelocytic leukemia cells. HL-60 cells were induced to differentiate into MP by 48 hr treatment with phorbol myristate acetate (170 nM) or into PMN with dibutyryl cyclic AMP (500 uM) or dimethylsulfoxide (1.3%). Cells were labeled for 3 hr with ^{32}P (250 uCi/ml) and phosphorylated proteins extracted and analyzed for phosphoamino acid content using thin layer electrophoresis following partial hydrolysis in 6N HCl. We found that total cellular protein content decreased during differentiation of the cells into PMN, but not MP. In addition, the relative amount of ^{32}P incorporated into cellular protein was less in both PMN and MP. We detected a decrease in the phosphoserine, phosphothreonine and phosphotyrosine content of the cellular phosphoproteins when compared to untreated controls. MP differentiation resulted in significantly greater reduction in phosphorylation of all three amino acids in proteins than did maturation into PMN. With all inducers, phosphorylation of tyrosine residues was decreased to a greater extent than was phosphorylation of serine or threonine residues. These data suggest that PMN and MP differentiation is associated with significant changes in cellular protein kinase and/or phosphatase activity. These data further indicate a role for protein tyrosine phosphorylation in regulating HL-60 cell differentiation. Supported by NIH Grant AI20183.

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THE IN VITRO PROLIFERATION OF PULMONARY ALVEOLAR MACROPHAGES FROM MICE UNDER MONOCYTOPENIA OR MONOCYTOSIS. Y. Oghiso Division of Comparative Radiotoxicology, National Institute of Radiological Sciences, Chiba 260, Japan

Pulmonary alveolar macrophages(PAM) obtained by lung lavage from the normal mice can proliferate in vitro with colony stimulating factors(CSFs) including M-CSF or GM-CSF. The present work was done to investigate whether or not such a growth kinetics of PAM in vitro would change either in a monocytopenic condition or under a monocytosis, compared to the normal steady state. The uptake of ^{3}H-TdR and colony formation by mouse PAM in a normal culture condition were not found during the incubation period of 14 days, whereas they were enhanced by addition of CSFs to the cultures. The expression of surface antigens on PAM, including Ia, Mac-1 and F4/80, was not, however, seen during the incubation in the presence of CSFs. Such in vitro proliferation of PAM was next examined in monocyte-depleted mice. During the period of 56 days after injection of a bone-seeking ^{89}Sr radioisotope, bone marrow stem cells and circulating monocytes were severely depleted, while splenic colony forming stem cells increased with an extramedullary hemopoiesis. PAM under this condition, however, showed almost equivalent ^{3}H-TdR uptake and colony formation in vitro by CSFs to the normal condition. On the other hand, when mice were implanted subcutaneously with NFSA fibrosarcoma cells, which have been found to enhance both of bone marrow and splenic hemopoiesis by producing GM-CSF, circulating granulocytes and monocytes increased in number. Any metastasis of tumors into the lung was not detected. In this condition, the recovery of PAM and their in vitro proliferative responses were not altered, compared to the normal or monocytopenic state. These findings indicate that there may exist precursor stem cells in the resident PAM population which can respond to the growth factors independently upon the level of circulating monocytes. We acknowledge a courtesy of Dr.R.K.Shadduck,University of Pittsburgh, for his gift of highly-purified M-CSF. This work was partly supported by a Special Cordination Fund for Promoting Science and Technology from Science and Technology Agency, Japan.

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RESIDENT PERITONEAL MACROPHAGES (MØ) ARE MAINTAINED BY LOCAL DIVISION. M.J. Melnicoff, T.C. Schmitt, P.K. Horan, and P.S. Morahan. Medical College of Pennsylvania, Phila., PA 19129; and Smith, Kline and French Laboratories, King of Prussia, PA 19406.

Resident peritoneal MØ of Balb/c mice were labeled in vivo by intraperitoneal injection of the green

fluorescent cell tracking dye, PKH-1. After immunofluorescence labeling with MØ specific monoclonal antibodies (Mabs), resident MØ were double labeled by both the green dye and the red (phycoerythrin) Mab tag. In the absence of induced inflammation, the number of PKH-1 labeled resident MØ did not decrease for at least 28 days in vivo; therefore, these cells were maintained without replacement by recruited monocytes.

To determine whether the resident MØ were dividing in vivo, a pulse of ^{3}H-thymidine was injected i.p. at 4-7 days after administration of the PKH-1 label. The animals were sacrificed one hour after the ^{3}H-thymidine pulse, and the peritoneal MØ were labeled with the Mab Mac-1. The resident MØ (Mab and dye labeled) were sorted on an EPICS V flow cytometer/sorter, and the fraction of ^{3}H-thymidine labeled MØ in each population was evaluated by autoradiography.

The unsorted peritoneal cells contained 29.6 ± 11.8% MØ (by differential cell counts), with a ^{3}H-thymidine labeling index of 3.3 ± 1.4% (N=6). Our present data show that the resident MØ fraction collected from the same animals after sorting contained 81.1 ± 8.2% MØ, of which 2.5 ± 1.9% were labeled by ^{3}H-thymidine. Three of the animals had detectable populations of recruited monocytes/MØ (Mab labeled only). The MØ labeling index of these recruited cells was 2.7 ± 2.3%. These data show that resident peritoneal MØ in the hematopoietically intact animal are dividing in vivo. (Supported by NCI CA3596 and NIH AI25751.)

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IDENTIFICATION OF PULMONARY MACROPHAGE POPULATIONS IN THE MOUSE. R. Crowell, B. Lehnert, C. Mold. (Spon: J. Bencer). Univ. NM, Alog., NM, Los Alamos Nat. Lab., Los Alamos, NM

Pulmonary macrophages (PM) exist in at least 2 compartments, the alveolar space and interstitium. We evaluated the contributions of each compartment to the total PM. Alveolar macrophages (AM) were recovered by bronchoalveolar lavage (BAL) and interstitial macrophages (IM) by lung mincing and digestion by collagenase. PM were identified morphologically and by their ability to phagocytize IgG opsonized sheep red blood cells (EA). The pulmonary circulation was perfused with PBS until the lungs turned white to eliminate blood monocytes. The lungs and trachea were excised, and BAL performed until return was less than 0.1ml. $2.3x10^6$ cells were recovered by BAL (83% viability) of which 87% were macrophages. 91.5% of AM phagocytized EA and 61% contained >4 EA/AM. Lung digestion by collagenase recovered $2.7x10^7$ cells (85.6% viability). When lungs were instilled intratracheally with EA prior to BAL and lung digestion, 4.4% of the lung digest cells contained EA suggesting these cells were AM not harvested by BAL. 8% of the cells ($2.1x10^6$ cells) in the lung digest had the morphologic appearance of blood monocytes, but failed to phagocytize EA. While 33.9% of the lung digest cells ($9.2x10^8$ cells) morphologically resembled PM, only 26.6% of these cells or 12% of lung digest cells phagocytized EA ($2.5x10^6$ cells). To determine if collagenase altered EA phagocytosis, BAL cells were incubated with collagenase under the conditions used for lung digestion. Only 71.2% of collagenase-treated AM phagocytized EA, and 40.2% contained >4 EA/AM. This decrease is insufficient to account for the discrepancy between cells identified morphologically and functionally as PM in the lung digest. We conclude that a subpopulation of mononuclear phagocytic cells resembling PM can be identified after exhaustive BAL and lung digestion. A significant portion (25%) of these cells are alveolar in origin. Accounting for these contaminants, the remaining cells comprise a larger IM compartment than previously reported in mice. The majority of cells in lung digest which morphologically resemble PM fail to ingest EA.

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CHARACTERISTICS OF HUMAN CD4 MONOCYTE(MØ) SUBSETS
G.Szabo,C.Miller,J.Wu & K.Kodys Univ. Mass. Medical Center, Worcester, MA 01655

Although human T lymphocytes and MØ bear the same Leu3 (T4,CD4) antigen, the function of CD4 expression on MØ in MØ-T cell interactions or inflammatory immune responses is uncharacterized. In this study, normal MØ depleted of T cells by E rosetting and separated into CD4 positive (26.87+ 5.7%) and negative (73.12+5.7%) MØ subsets by panning with anti CD4 ab were assessed for presentation of tetanus toxoid. Antigen presenting capacity (APC) was expressed in cpm over control. $CD4^+$ MØ subset's APC activity was increased concomitant to decreased production of prostaglandin E_2 (PGE_2 as measured by ELISA in ng/10^6 MØ) when compared to the $CD4^-$ MØ subset. The reduced APC function of $CD4^-$ MØ may not be secondary to their greater PGE_2 production via possible downregulation of Class II antigens by PGE_2. Even in the presence of indomethacin (10^{-6}M) $CD4^-$MØ APC was reduced from that of $CD4^+$ MØ.

	$CD4^+$	$CD4^-$	$CD4^+$	$CD4^-$
PGE_2	5.6	10.0	6.4	8.2
APC	17,321	8,357	44,886	35,411
APC+indo	30,891	15,891	55,957	48,512

Plasminogen activator capacity of the subsets was similar ($CD4^+$:26.8,$CD4^-$:29.2% specific fibrinolysis). Secretion of the inflammatory monokine tumor necrosis factor (TNF, ng/10^6 MØ in L-M bioassay) and interleukin-1 (IL-1 stimulation index in D.10 bioassay) were also similar in the subsets after 100U/10^6 MØ IFNγ plus 20μg/ml muramyl dipeptide (MDP) stimulation.

	$CD4^+$	$CD4^-$	$CD4^+$	$CD4^-$
TNF unstim.	1.74	1.44	0	0
IFN+MDP	6.47	7.46	4.87	3.29
IL-1 unstim.	5.64	7.04	2.05	2.05
IFN+MDP	29.90	26.60	6.90	7.02

These data suggest, that the presence of CD4 antigen on MØ could be related to the better APC of $CD4^+$ MØ subset to T cells. Since it is the $CD4^+4B4^+$ T cells that primarily proliferate to soluble antigens presented by MØ, CD4 ag complex may have a role in MØ activation of T helper cells.

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HUMAN MONOCYTE HETEROGENEITY DEFINED BY HLA-DR EXPRESSION DOES NOT CORRELATE WITH OXIDATIVE BURST CAPABILITY. G.T. Spear, L.C. Rothberg and A.L. Landay. Rush University, Chicago, IL 60612.

Monocytes from any one blood donor exhibit a wide range of HLA-DR expression with the difference between low and high HLA-DR expressing cells being as great as 10-fold. This study determined if the variability in HLA-DR expression on monocytes correlated with oxidative burst capability by cells stimulated by heat-aggregated IgG (HagIgG), phorbol myristate acetate (PMA) and calcium ionophore A23187 (ionophore).

Freshly obtained cells were loaded with dichlorofluorescin diacetate, stimulated, and then stained with phycoerythrin-labeled anti-LeuM3, anti-HLA-DR or control monoclonal antibodies (mAb). Two-color flow cytometry was used to determine changes in green fluorescence corresponding to oxidative product formation by cells, which stained only with the red mAb (monocytes). Forward and 90° light scatter were also used to ensure that only monocytes were detected.

The oxidative capacity of total Leu-M3 and total DR-stained cells was identical, indicating that these markers measured similar functional populations. While monocytes expressing high levels of HLA-DR and low levels

differed substantially, (average difference = 65 channels), changes in oxidative capability were only 2 channels for HagIgG, 9 for PMA and 14 for ionophore. Other myeloid markers exhibiting less heterogeneity on normal monocytes than HLA-DR gave similar results. Thus, monocytes which are very different in HLA-DR expression are very similar in oxidative burst capability.

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BIOACTIVITY OF THE INSULIN RECEPTORS (IR) ON SPLENIC MACROPHAGES (Mø) IN MICE. A.P. Bautista, D.J. Fletcher & A.Volkman. School of Medicine, East Carolina University, Greenville NC 27858.

The presence of IR on splenic Mø suggests that insulin may be important for the regulation of the biological activities of these cells. The bioactivity of IR in C. parvum (CP) and 89Sr-monocytopenic mice, in which there is increased proliferation of splenic Mø progenitors was studied. CBA/J mice received 4 uCi/g bw 89SrCl2 IV. On day 3, CP was given IP into each mouse at a dose of 60 mg/Kg bw. Control mice received non-radioactive 88SrCl2 and 0.15 M sterile NaCl. Insulin binding assays were performed using 125-I-insulin as tracer.Mø colony forming cells(M-CFC) were determined using a 2-layer agar technique.Results show progressive elevation of specific 125-I-insulin binding from days 2-10, when it was significantly greater (P<0.01) in CP-mice (12.5 ± 1.18 pg/1 x 10^6 Mø) than in the parallel controls (6.25 ± 0.8 pg). The increase in 125-I-insulin specific binding was due to an elevation of both high and low affinity sites without any change in binding affinities, and with no significant alterations in plasma insulin. In monocytopenic mice, 125-I-insulin binding to splenic Mø was greater (10 ± 0.9 pg;P<0.05) than the control, and was further increased (P<0.05) to 14 ± 1.2 pg after the administration of CP. Although insulin alone could not induce the formation of M-CFC by splenic cells, it was associated with a dose-dependent modulation of M-CFC in control (P≥0.05) and CP mice (P<0.01). Insulin at more than 1 uM was associated with reduced M-CFC in control and CP groups. M-CFC (4.57 ± 0.97/ 20,000 nucleated cells) in the spleens of control mice were significantly increased in the presence of 1 nM insulin, than without (3.0 ± 0.86 ; P<0.01). In the CP-treated group similar responses were observed : with insulin, M-CFC was 169.4 ± 38 and without 113 ± 27 (P<0.01). These observations thus suggest that insulin at optimum concentrations may augment the proliferative capacity of splenic M-CFC.

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MORPHOLOGICAL CHANGE OF B CELL AND MONOCYTE IN VITRO WITH PMA (4β-PHORBOL 12-MYRISTATE 13-ACETATE). M.Matsuda, M. Ishikawa, A.Masunaga, M.Narabayashi, H.Hashimoto and Y. Imai.
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There are still some debates on the nature of various accessory cells concerning the immune reactions. Recently, peripheral B lymphocyte has been reported to transform into interdigitating cell (IDC) mainly by morphological observation. In this paper, the changes of morphology and some cell markers of B cell and monocyte were studied in vitro culture in the medium (RPMI1640, 20%FCS) containing 10 ng/ml of PMA. Two B cell lines (TA : derived from reactive lymph node and 154 : from EBV infected B cell) as well as peripheral B lymphocyte and one monocyte line (YS :from AMMoL) were examined. Each cell showed an adherent character and became to reveal cytoplastic processes on 2nd culture day. They extended their processes,thereafter, making a "dendritic form". This morphologic alteration became to be most conspicuous on 4-5th culture day. Thin and elongated dendritic processes were remarkable in the cells of peripheral B cell, TA and 154, in the contrast with shorter and wider ones in YS cells. As the cell markers of this period, CD20 and CD21 were positive in each cells other than YS. All cells of examined cell lines were positive for HLA-DR. There were positive cells for CD1 and LeuM3 partly in YS and IL2-R in all cells except for YS. Positive reaction of DRC-1(anti-follicular dendritic cell) was seen in TA and 154 cells. Some of YS cells showed characteristically positive for lysozyme, S100 protein and Lag (anti-Langerhans cell).

These results may suggest that B cells altered their morphology to dendritic form with PMA still reserving the cell markers of B cell characters. On the other hand, monocytoid cell of YS was capable of transforming into IDC on the viewpoint of its morphology and cell markers as well.

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EFFECTS OF BONE MARROW SUPPRESSION WITH ^{45}Ca ON MONOCYTES AND MACROPHAGES (MØ). A. Volkman and Y. Shibata. East Carolina University School of Medicine, Greenville, NC 27858-4354.

Responses of mice to P. acnes vaccine (PA) ip include the elicitation of monocytes into the peritoneal sac and the induction of PGE_2-secreting suppressor MØ (PGSM) in the spleen. Bone marrow ablation with the bone-seeking isotope, ^{89}Sr (T1/2-50 days, Eβ-.56Mev), in mice results in profound monocytopenia and a 10x increase in splenic M-CFC but loss of the monocyte elicitation and PGSM responses to PA. This and other observations suggested bone marrow dependence of the PGSM response but a dissociation of elicitation and PGSM induction was found in congenitally monocytopenic Sl/Sl^d mice (Shibata, Y. and Volkman, A. JI, 135, 3905, 1985). To pursue this point, $^{45}CaCl_2$ (T1/2-164 days, Eβ-.077) was given iv to 8-12 wk old CBA/J mice, 4μCi/g bw; $^{40}CaCl_2$ was used for controls. At 14 days blood monocytes were 14% control in unstimulated mice, 33% in PA (56 mg/kg, ip day 7) mice, and 29% in thioglycollate (TG) (4 day) mice. Monocyte counts were less depressed in all groups on day 28 but still about 50% below controls on day 44. Neither PA nor TG was able to elicit monocyte-MØ in day 14 mice but progressive return was seen in 28 and 44 day samples. M-CFC day 14: marrow, 25% (no PA) and 12% (PA) of controls; spleen, 6x (no PA) and 3x (PA) controls. Unlike ^{89}Sr mice, however, ^{45}Ca treatment did not impair release of PGE_2 from PA-induced PGSM whether spontaneous or enhanced with zymosan, Ca^{2+} ionophore A23187 or phorbol ester. The observed dissociation between monocytopenia and failed elicitation on the one hand and unimpaired PGSM activity on the other suggests that blood monocytes are not precursors of PGSM. Since splenic M-CFC are elevated in PGSM-impaired ^{89}Sr-mice and in PGSM-unimpaired ^{45}Ca-mice their role in this function remains uncertain. The different effects of ^{89}Sr and ^{45}Ca on PGSM may be due to differences in Eβ suggesting that monocytopoiesis is a more radiosensitive path than PGSM formation. (Supported in part by NIH grant AI17162.)

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INDUCTION AND CHARACTERIZATION OF HUMAN MONOCYTE-MACROPHAGE-DERIVED MULTINUCLEATED GIANT CELLS IN IN VITRO CULTURE. N. Hassan and S. Douglas. Division of Allergy-Immunology-BMT, Children's Hospital of Philadelphia, Univ. of Pennsylvania Medical School, Phila., PA 19104.

Human blood monocyte-macrophage-derived multinucleated giant cells are observed in several pathological conditions including inflammatory diseases, foreign body reactions, cancer, and also in viral infections such as the acquired immunodeficiency syndrome. Although multinucleated giant cells have been described for a century (Metchnikoff, 1888) the mechanism of their formation and functions are unknown. Several studies have described the induction of fusion of monocytes and macrophages using gamma interferon (Weinberg et al., Proc. Natl. Acad. Sci. USA 81:4554, 1984), 1,25-dihydroxyvitamin D3 (Abe et al., Proc. Natl.

Acad. Sci. USA 81:7112, 1984), and Interleukin-4 (McInnes et al., J. Exp. Med. 167:598, 1988). We have demonstrated the fusion of human blood monocyte-derived macrophages in vitro culture by phorbol myristate acetate (PMA) with the induction and formation of multinucleated giant cells (Clinical Research 36(3):440A, 1988). PMA (10-80nM) induces fusion of human monocyte-derived macrophages with 35-85% fusion rate. These giant cells form in cultures within 12-24 hours after the addition of the stimulus, and they are 200-400 um in diameter and contain 15-30 nuclei. The addition of gamma interferon (1000 U/ml) to the cells prior to PMA (10nM) enhances fusion rate of the cells from 35% to 100%. In comparison to monocyte-derived macrophages, PMA induced multinucleated giant cells are characterized by decreased intracellular acid phosphatase levels, decreased or absent oxidative burst activity by nitroblue tetrazolium reduction test, normal tumor cytotoxicity using the CEM leukemia cell line as the target cell, and decreased cell membrane ruffling with scanning electron microscopy. We conclude that the human blood monocyte-macrophage-derived multinucleated giant cells have different functional capabilities, as compared to monocyte-derived macrophages, and that these cells may play an important role in tumor cell killing. (Supported in part by grants from NIH HL-27068, NS-17752, AMFAR 161, and MS Society 1919A-1).

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Differential production of tumor necrosis factor (TNF), macrophage colony stimulating factor (CSF-1) and interleukin 1 (IL-1) by human alveolar macrophages. Susanne Becker, Robert Devlin and Stephen Haskill*Environmental Monitoring and Services, Inc., U.S. Environmental Protection Agency, Chapel Hill, NC 27516.

Human alveolar macrophages (AMO) have been investigated for their ability to produce the three monokines, TNF, CSF-1, and IL-1. No TNF activity was found in supernatants of unstimulated AMO cultured for 20 hours, although TNF mRNA was detected in the cells by Northern blot analysis. Stimulation of the cells with LPS induced production and release of high levels of TNF into the culture supernatant. Increased levels of TNF mRNA was detectable at 90 min after LPS stimulation by dot blot analysis, reaching peak expression between 4-8 hours, and declining thereafter. TNF activity peaked at approximately 8 hours in the AMO sups. After 24 hours TNF production had ended. Compared to autologous monocytes the AMO produced 5.7 times more TNF on per cell basis (activity in 20 h supernatants). AMO expressed high and low mol. weight CSF-1 mRNA and active protein was recovered in supernatants upon culture. LPS stimulation of the cells slightly reduced both mRNA levels and amount of factor in the supernatants. In contrast to the AMO, monocyte production of CSF-1 was enhanced by LPS. CSF-1 production by both monocytes and AMO continued for at least 48 hours of culture. Low levels of IL-1 mRNA was present in all tested AMO preparations. LPS stimulation induced increase in IL-1 mRNA within 90 min., mRNA levels peaked between 12 and 20 hours, and stayed high for at least 42 hours. However, while the AMO expressed high levels of IL-1 mRNA upon stimulation with LPS, biologically active IL-1 was detected only in a fraction of the AMO supernatants. These results show that the production of monokines CSF-1, TNF, and IL-1 are differentially regulated in alveolar macrophages.

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INDUCTION OF DIFFERENTIATION IN HUMAN U-937 HISTIOCYTIC LEUKEMIC CELLS BY DIBUTYRYL CYCLIC ADENOSINE-3',5'-MONOPHOSPHATE (dBcAMP). A.J. Beavis, J.D. Laskin, A.A. Sirak, S.M. O'Connell and D.L. Laskin, Rutgers University and UMDNJ-RWJ Medical School, Piscataway, NJ 08854

Treatment of U-937 cells with the cyclic nucleotide analog dBcAMP induced these cells to differentiate. DBcAMP produced a dose- and time-dependent inhibition of cell growth reaching a maximum after 48 hr treatment with 500 uM. At this concentration, dBcAMP had no effect on cell viability. Flow cytometric analysis of dBcAMP-treated cells revealed a time-dependent decrease in the number of cells in S-phase of the cell cycle, with a concurrent increase in cells in the GO/G1 phase. Treatment of U-937 cells with dBcAMP also decreased cell size, produced alterations in the cell membranes and decreased nuclear-to-cytoplasmic ratios. DBcAMP also induced the appearance of f-met-leu-phe (FMLP) receptors and the monocytic/granulocytic cell surface markers MY8 and MAC-1 on U-937 cells, but decreased levels of HLA-DR expression. Treated cells did not express the monocyte-specific markers, Mo2 or MY4. In addition, neither dBcAMP-treated nor untreated U-937 cells stained for non-specific esterase nor did they adhere to culture dishes. Using flow cytometry we also found that differentiated U-937 cells produced H_2O_2 and O_2^-. FMLP was more effective in stimulating H_2O_2 production by the cells than was phorbol myristate acetate. However both responses were inhibited by catalase. DBcAMP-treated cells also produced greater amounts of O_2^- than did untreated cells, as determined by nitroblue tetrazolium and ferricytochrome C reduction. These studies indicate that dBcAMP induces partial differentiation of U-937 cells. Furthermore, these differentiated cells display characteristics typical of granulocytic cells. Supported by NIH grant AI20183.

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INDUCTION OF MACROPHAGE DIFFERENTIATION OF THE HUMAN PROMYELOCYTIC CELL LINE HL-60 AS DETERMINED BY FUNCTION AND IMMUNOCYTOCHEMISTRY. R.H.J.Beelen, I.L.Eestermans*, H.J.Bos*, and G.J.Ossenkoppele. Departments of Haematology and Cell Biology*, Free University Hospital and Medical Faculty*, Amsterdam, The Netherlands

Our earlier studies (Eur J Clin Invest 17,1987,63) on human bone marrow cultures have shown that the endogenous peroxidatic activity (PA) is a very helpful tool to identify the immature and mature cells of the mononuclear phagocyte and granulocyte series. In this study we investigated the PA pattern of the human promyelocytic leukaemia line HL-60 which is known to differentiate into macrophages or granulocytes upon induction by chemicals. The results showed clearly promyelocytes and blasts (PA in the Golgi system, granules and RER) in the control (no inducer) as well as after the addition of Ara-C. Vit. D (1.25 dihydroxy Vit. D3) induced the differentiation into monocytes (PA only in lysosomes), while Vit. A (retinoic acid) resulted in a differentiation of granulocytes. Most remarkably DMSO gave a very pronounced differentiation into both very mature granulocytes as well as mature resident macrophages (PA only in RER). The results, with respect to the ultrastructural cytochemistry, fitted very well in the functional characteristics of these cells, since after induction with Vit. D and also DMSO the cells showed an enhanced Fc receptor activity which correlated with a clear functional antibody dependent phagocytosis (ADP). No ADP was found in the control or after induction with the other 2 chemicals. In agreement with this finding both Vit. D and DMSO resulted in a chemotactic activity to the chemoattractant FMLP comparable to normal human macrophages. Finally Vit. D was found to induce the expression of HLA-DR on this cell line, which also resulted in the capacity of these cells to function as antigen presenting cells in a MLR.

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SURVIVAL ENHANCEMENT AND HEMOPOIETIC REGENERATION FOLLOWING RADIATION EXPOSURE: THERAPEUTIC APPROACH USING GLUCAN, A MACROPHAGE-ACTIVATOR, IN COMBINATION WITH GRANULOCYTE-COLONY STIMULATING FACTOR. M.L. Patchen, T.J. MacVittie, B.D. Solberg, L.M. Souza. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145 and AMGen, Thousand Oaks, CA.

We have previously demonstrated glucan enhances survival in irradiated mice. Macrophage-mediated resistance to postirradiation opportunistic pathogens and accelerated hemopoietic stem cell regeneration mediate this effect. Granulocytes are also known to be important in defense against infection. Because of this, it was hypothesized that the use of granulocyte colony stimulating factor (G-CSF) in combination with glucan may further enhance survival by selectively directing stem cells toward granulocytic differentiation. C3H/HeN mice were exposed to whole-body irradiation and administered soluble glucan (250 mg/kg i.v. at 1 h postexposure), recombinant human G-CSF (2.5 ug/day s.c. on days 3-12 postexposure; AMGen), or both agents. In addition to survival-enhancing effects of these treatments (based on 30-day survival following 8 Gy irradiation), femoral and splenic cell and granulocyte progenitor (G-CFC) contents were determined on days 4, 7, 10, 12, 14, 17 and 20 postexposure to 6.5 Gy. Greatest survival was observed in mice treated with G-CSF in combination with glucan. Likewise, greatest hemopoietic regeneration was observed in mice receiving both agents. For example, on day 14 postexposure, mice treated with saline, G-CSF, glucan, or both agents, respectively, exhibited 56%, 57%, 69% and 74% of normal bone marrow cellularity and 29%, 38%, 99% and 146% of normal splenic cellularity. At this same time, CFC values in saline, G-CSF, glucan, or combination treated mice, respectively, were 8%, 10%, 14% and 52% of normal bone marrow values and 2%, 93%, 22% and 1443% of normal splenic values. Peripheral WBC counts were also, respectively, 18%, 16%, 36% and 94% of normal with combination-treated mice also exhibiting more granulocytes than mice receiving single treatments. These results 1) reconfirm glucan's survival enhancing and hemopoietic effects in irradiated mice, 2) illustrate the ability of G-CSF to enhance survival and to accelerate hemopoietic recovery in irradiated mice, and 3) suggest that agents acting via different immuno/hemopoietic mechanisms may be used in combination to further enhance survival and selectively accelerate hemopoietic repopulation.

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PURIFICATION OF HEMATOPOIETIC PROGENITOR CELLS FROM HUMAN PERIPHERAL BLOOD P. Law, D. Dooley, P. Alsop and L. Haiber (Spon: M. Patchen). American Red Cross, Rockville, MD 20855.

The purpose of this study was to develop a technique for the purification of hematopoietic cells from human peripheral blood (PB). Starting with plateletapheresis residues, 3 to 6 x 10^9 PB mononuclear cells (MNC) were isolated by centrifugation on ficoll-sodium diatrizoate density gradients (1.077 g/ml) in the Haemonetics V50 blood processor. E-rosetting followed by fractionation on 4 layer Percoll density gradients was used to remove 95-98% of T lymphocytes (T^- MNC). Monocytes were removed by treatment with 5 mM phenylalanine methyl ester, yielding T^-M^- MNC. The cell suspension was than incubated with 100 ug/ml Campath-1M* monoclonal antibody (generously provided by Wellcome Biotech) for 30 min at room temperature. Heparinized human plasma was added afterwards. Campath-1M is a rat IgM which is demonstrated to be cytolytic for T cells, B cells and some monocytes in the presence of human complement. After Campath treatment, the cells were centrifuged through 25% Percoll. Dead cells and debris floated to the top of the gradient. Excessive cell loss due to non-specific clumping of dead and live cells was observed when the Percoll centrifugation step was omitted. The final cell product, called null cells, contained 1.17±0.31% (Mean±SEM, N=8) of the original MNC. Viability was 80.3±2.9%. An average of 40.1±6.1 CFU-GM (N=8) and 42.9±10.8 (N=4) BFUe were cultured from 5 x 10^3 null cells. CFU-GM and BFUe constituted at least 1.47±0.17% of the null cell population. CFU-GM were enriched 170-fold (range: 18 to 440-fold) from MNC while BFUe enrichment averaged 304-fold (range: 217 to 391-fold). Accessory cells that were inhibitory to CFU-GM growth were apparently removed during the purification steps, as CFU-GM recovery averaged 170±121% (N=8; range 25 to 1020%). For comparison, MNC and T^- MNC were incubated with Campath-1M and complement. Enrichment and recovery of progenitor cells were both lower than that achieved by Campath-1M treatment of T^-M^- MNC. We conclude that Campath-1M produced the best result with PB when applied to T^-M^- MNC. The procedure yields a cell product highly enriched in hematopoietic cells which will be suitable for in vitro studies of hematopoiesis and its regulation.
*Trademark of Wellcome Foundation Limited.

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EFFECTS OF INTERLEUKIN-1 (IL-1) ON GRANULOCYTE AND MACROPHAGE PROGENITOR CELLS IN NORMAL AND IRRADIATED MICE. G.N. Schwartz, M.L. Patchen, and T.J. MacVittie. Armed Forces Rad. Res. Inst., Bethesda, MD 20814 and Amer. Red Cross, Rockville, MD 20855

Neta et al. (J. Immunol. 139:1861, 1987) demonstrated an increased proliferation, in response to GM-CSF, of bone marrow cells from mice administered IL-1 20 hours earlier. In the present studies, the effect of rIL-1 injection on the number of granulocyte-macrophage colony-forming cells (GM-CFC) and high proliferative potential colony-forming cells (HPP-CFC) was investigated in normal and irradiated mice. Twelve week old B6D2F1 female mice were administered 150 or 5 ng human recombinant IL-1-alpha (Hoffman La Roche) or 0.5 ml pyrogen free saline by a single intraperitoneal injection. Some mice were also exposed to 0.5 Gy Co^{60} radiation 20 hours after injection of saline or IL-1. A significant increase in neutrophils and a decrease in lymphocytes in peripheral blood were observed within 2 hours after injection of 150 or 5 ng IL-1. Cell mobilization from the marrow was still evident 20 hours after injection. Neutrophils were 22±3.2%, 35±3.9%, and 42±6.2%, respectively for saline, 5 ng rIL-1, and 150 ng IL-1 injected mice. Compared to saline injected mice, marrow cellularity was decreased to 84±5.2% (5ng) and 59±3.2% (150ng) 20 hours after injection. Also, the number of GM-CFC and HPP-CFC was decreased in bone marrow from IL-1 injected mice. For example in 1 study, GM-CFC/femur were 70% (5ng) and 30% (150ng), and HPP-CFC/femur were 81% (5ng) and 70% (150ng) of the number in saline injected mice. These values were normal by 3 days after injection. Three days after irradiation, both GM-CFC and HPP-CFC per femur were 1.6 fold higher in mice pretreated with 150 ng IL-1 than in saline or 5ng IL-1 injected mice. These results demonstrate that stimulation of granulopoiesis after irradiation is dependent on rIL-1 dose and may be in response to changes that occur due to the mobilization of cells from the bone marrow early after the injection of IL-1.

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QUANTITATIVE MODEL OF MACROPHAGE LINEAGE PROLIFERATION IN MICE. J.P. Novak, E. Skamene* and F. Gervais,* Institut de recherche d'Hydro-Québec, Varennes, Québec, Canada J0L 2P0 and *Montreal General Hospital Research Institute, Montreal, Quebec, Canada H3G 1A4.

An analysis and synthesis of the experimental evidence pertinent to monocyte-macrophage kinetics have been performed with regard to global qualitative and quantitative description of the proliferation of the monocyte precursors in murine bone marrow. Subsequently, two versions of the mathematical model have been suggested: First version consists of two dividing generations of granulocyte - macrophage colony forming cells (GM-CFC) and two dividing generations of macrophage colony forming cells (M-CFC), differentiating into promonocytes (PM) and finally monocytes (MC). Second version consists of two generations of GM-CFC, and three generations of M-CFC, followed by PM and MC. Kinetic constants have been estimated from available data whenever possible and unknown coefficients determined by comparison of the calculated and measured quantities. A critical evaluation of the cell kinetics with the help of the models have shown that the requirements imposed by normal steady state conditions strongly suggest existence of a large quiescent pool

(G_0 compartment) of M-CFC. The models have been used to simulate ^{3}H-thymidine pulse labeling assay and both alternatives yielded a satisfactory agreement with published experiments. The main contribution of the models at this stage of development is the formulation of the quantitative interrelationships among seemingly independent experimental quantities and the elucidation of the proliferation mechanism as an integrated, self-consistent phenomenon.

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Identification of the Regulatory Signals Controlling the Proliferation and Differentiation of Mouse Hematopoietic Stem Cells. Brown, RL*. Keller, J*. Quality Biological, Inc., Gaithersburg, MD; Biological Carcinogenesis Development Program, Program Resources, Inc., Frederick Cancer Research Facility, Frederick, MD.

The purpose of this study was to identify the regulatory molecules which control the proliferation and differentiation of murine hematopoietic stem cells. Using a soft agar assay containing serum-free medium and recombinant granulocyte-macrophage colony stimulating factor or purified Interleukin-3 the role specific regulatory molecules play in proliferation and differentiation of murine granulocytes and monocytes was analyzed. For erythroid differentiation and proliferation murine spleen cells were analyzed in a fibrin clot assay containing serum-free medium and erythropoietin. In both cases the basal medium was Iscove's Modified Dulbecco's Medium supplemented with the serum-free components or fetal bovine serum (FBS). In both assays the serum-free components included bovine serum albumin, cholesterol, insulin and transferrin. Granulocyte and macrophage colony formation was found to be dependent on only transferrin and the appropriate growth factor. In the presence of growth factor colony formation was equivalent in basal medium containing either the serum-free components or FBS. Erythroid colony formation (CFU-E) was also found to be dependent on the appropriate growth factor (erythropoietin) and transferrin. Basal medium containing only transferrin and erythropoietin supported erythroid colony formation to within 70-80% of basal medium containing FBS and erythropoietin. In conclusion, the proliferation of mouse granulocytes, monocytes, and erythroid stem cells appears to be regulated by at least two external signals transferrin and the appropriate growth factor. The role these stimuli play in the human versus the murine hematopoietic system will be presented.

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IMMUNOHISTOCHEMICAL STUDY OF FcεR IN LYMPH FOLLICLE AND FOLLICULAR LYMPHOMA. A. Masuda*, T. Kasajima* and M. Kojima**, * Tokyo Women's Medical College, Kawadacho, Shinjuku-ku, Tokyo, Japan, ** Mito Saiseikai Hospital, Futabadai, Mito, Japan.

Low affinity IgE Fc receptor (FcεR), which has a function of the regulation of IgE production, has been described on a variety of hematopoietic cells. On the other hand, IgE usually distributes in germinal centers (GCs) of lymph follicles (LFs) in the granuloma of eosinophilic lymphfolliculoid granuloma (Kimura's disease) which is characterized by hyper IgE and eosinophilic granuloma. In GCs of Warthin's tumor and tonsillitis, IgE is sometimes detected. In the IgE immune response, follicular dendritic cell (FDC) may play an important roles. Present author examined the distribution of FcεR in the LF of lymph node, extranodal LFs in Kimura's disease, Warthin's tumor, thyroid disorder and tonsil, and follicular lymphoma by immunohistochemical methods with specifically reacting monoclonal antibody to FcεR (H107). In the GCs of the LFs in the tissue examined, FcεR distributes with lacy network pattern which are proven electron microscopically to coinside mainly with the surface of FDCs. FcεR in the GCs was detected irrespective of positivity of IgE. In general, FcεR was positive in the light zone and not in the dark zone which was positive area of DRC1 and complement receptors. IgE positive GCs in Kimura's disease and Warthin's tumor were positive for FcεR in their entire portion. Moreover, IgE positive GCs revealed positive reaction for FcεR more intensively than IgE negative GCs. FcεR positive lymphoid GC cells were observed, besides FcεR positive cells were inclined to increase in IgE positive GCs. Mantle zone lymphocytes were positive for FcεR. In follicular lymphoma, reticular staining of FcεR was detected in all the cases. The FcεR positive area was smaller than DRC1 positive area, and the difference of the positive area between FcεR and DRC1 showed variety among the cases. These findings indicate that FcεR on FDCs has close relation to IgE immune response and also was a indicater for functional phase or differentiation of FDCs.

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ENHANCEMENT OF HUMAN MONOCYTE CYTOTOXICITY BY MULTIPLE SPECIES OF INTERFERON - ALPHA. D. Webb, K. Zoon, D. Zur Nedden, and T. Gerrard (Spon: J. Roth) FDA, Bethesda, MD 20892

Twenty-one purified species of human interferon (IFN)-alpha species were isolated from Sendai virus stimulated Namalwa cells and evaluated for their ability to enhance monocyte-mediated cytolytic activity. There were significant differences among the various IFN-alpha species in their ability to enhance monocyte cytotoxicity, ranging from no enhancement to threefold enhancement. There was very little direct killing of the target cells by the IFN-alpha species in a 72-hour assay, indicating that the IFN-alpha species were inducing tumoricidal activity in the monocytes. Some IFN-alpha species were superior to IFN-gamma in enhancing monocyte cytotoxicity to the human melanoma cells, A375. Monocytes treated with IFN-alpha species lysed A375 cells at a faster rate than IFN-gamma treated monocytes. Monocytes required 4 hours preincubation with either the IFN-alpha species or IFN-gamma to induce maximal cytotoxicity. Unlike the A375 cells, HT-29 cells, a human colon carcinoma cell line, were more susceptible to killing by the IFN-gamma treated monocytes than by IFN-alpha treated monocytes. This may indicate that IFN-alpha and IFN-gamma induce different mechanisms of monocyte tumoricidal activity. In general, there was a positive correlation between the monocyte enhancing abilities of the various IFN-alpha species and their antiviral activities. The results indicate that IFN-alpha is a potent activator of monocyte tumoricidal activity, but substantial differences exist among the different IFN-alpha species. Furthermore, valid comparisons between the monocyte activating actions of IFN-alpha and IFN-gamma must consider the particular IFN-alpha species and the target cells used.

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INTERLEUKIN-4 INDUCES TUMOR CYTOTOXICITY IN THE ABSENCE OF DETECTABLE TUMOR NECROSIS FACTOR MESSENGER RNA. R. H. G. Wolff, L. S. D. Anthony, R. M. Crawford, C. A. Nacy and M. S. Meltzer. Walter Reed Army Inst Res., Washington, DC, 20307-5100

Interleukin-4 (IL-4) activates murine macrophages to express surface Ia antigen and tumoricidal activity. To further characterize effects of IL-4 on macrophage function, we evaluated production of tumor necrosis factor-α (TNF)-specific mRNA by Northern dot blot analysis. Several reports document TNF as a major lytic mediator of the activated, cytotoxic macrophage. Proteose peptone-elicited peritoneal macrophages from C3H/HeN mice were cultured with IL-4 or interferonγ

(IFNγ), with or without bacterial endotoxic lipopolysaccharide (LPS). Total cellular RNA was immobilized on nitrocellulose, then probed with ^{32}P-dCTP-labelled TNF DNA. TNF-specific mRNA was not detected in control macrophages cultured in medium alone. Cells treated with either IFNγ or IL-4 alone also did not express an appreciable quantity of TNF mRNA, whereas LPS by itself stimulated significant TNF mRNA levels. This response was observed after several hrs in culture and was short-lived. Addition of IFNγ to cultures treated with LPS markedly increased levels of TNF mRNA. In contrast, the amount of TNF mRNA observed in macrophages treated with both IL-4 and LPS was not significantly different from that of cells stimulated with LPS alone. These data suggest that IL-4 does not stimulate TNF mRNA expression, and IL-4-induced tumor cytotoxicity may not be mediated by TNF.

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MACROPHAGE RESISTANCE TO INFECTION WITH *Leishmania major* : INDUCTION BY TUMOR NECROSIS FACTORα. Miodrag Belosevic and Carol A. Nacy. Univ. of Alberta, Edmonton, Canada and Walter Reed Army Inst. of Res., Washington, DC 20307-5100

Macrophages from C3H/HeN mice pretreated with lymphokines (LK) *in vitro* develop the capacity to resist infection with amastigotes of *L. major*, an obligate intracellular protozoan parasite. The factors responsible for induction of this activated macrophage effector activity chromatograph as 55, 35, and 20 kD activity peaks on sizing gels. The first two, M_r 55,000 and 35,000, require interferonγ (IFNγ) for activity; the M_r 20,000 molecule does not. Tumor necrosis factorα (TNF, M_r 17,000) is increasingly recognized as a regulatory cytokine for a number of immune reactions. Resistance to infection developed in cultures of macrophages treated with 30 to 100 U/ml TNF: 45% fewer cells became infected in these cultures compared to medium-treated controls. The effect of TNF was on macrophages rather than directly on parasites, since amastigotes incubated 2 to 24 hr in 100 U/ml TNF were not killed. Activity of TNF was markedly synergized by the presence of 50 U/ml IFNγ: up to 80% fewer infected macrophages were observed in cultures treated with both cytokines. This synergy occurred across a broad dose response for TNF: the concentration of TNF required for 50% maximal resistance to infection was 37 ± 8 U/ml by itself, and 12 ± 4 U/ml in the presence of IFNγ. IFNγ alone did not induce resistance to infection. The synergistic interaction of TNF and IFNγ could not be reproduced in cultures treated with TNF and other cytokines (IL-1, IL-2, IL-4, GM-CSF, IFNα/β). The capacity of TNF to induce resistance to infection was also not augmented by 100ng of LPS. Macrophages from C3H/HeJ mice failed to develop resistance to infection in the presence of TNF, with or without IFNγ. These data suggested that TNF itself was involved in the mechanism of resistance to infection, although we could not demonstrate that exogenous TNF was toxic to the parasite. To analyse this possibility, we activated macrophages with TNF with or without IFNγ for 20 hr, washed away the cytokines, and added anti-TNF antibodies to the cultures prior to addition of parasites. Resistance to infection was totally abrogated by anti-TNF antibody, but not by an irrelevant antibody of the same isotype. Thus, TNF induced resistance to infection, a potent antimicrobial effector response of activated macrophages, and was also involved in the effector phase of the reaction that it induced. The activity of TNF for induction of resistance to infection was synergistically enhanced by IFNγ.

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PERITONEAL CELLS OF CAPD PATIENTS, AND ESPECIALLY THE NON ADHERENT SUBPOPULATION, ARE GOOD STIMULATORS OF A MHC CLASS-II ANTIGEN DEPENDENT ALLOGENEIC MIXED LEUCOCYTE REACTION. H.J.Bos, E. de Lang, J.C. de Veld and R.H.J.Beelen*. Departments of Cell Biology and Haematology*, Medical Faculty and University Hospital*, Free University, Amsterdam, The Netherlands

The expression of class-II MHC antigens on macrophages in the rat, mouse and in the human system has been shown to be essential for antigen presentation. We examined the role of MHC class-II antigens in an allogeneic mixed leucocyte reaction (MLR) with (subpopulations of) peritoneal cells (80% macrophages) from continuous ambulatory peritoneal dialysis (CAPD) patients and healthy volunteers undergoing laparoscopy.
Peritoneal cells of healthy volunteers induces a proliferative response which is comparable with peripheral blood monocytes. However, peritoneal cells obtained from CAPD patients show a two- to tenfold higher proliferative response in the MLR. Especially the non-adherent cells (NAC) from CAPD patients, which contain dendritic-like cells (acid phosphatase in a spot near the nucleus), are very potent stimulators of allogeneic lymphocytes. The addition of excess anti HLA-DR monoclonal antibodies (MoAb) did block this proliferation induced by peritoneal macrophages, whereas HLA-DQ MoAb not or only partially blocked this proliferation. Peritoneal cells of some CAPD patients, some healthy volunteers and the subpopulations enriched with dendritic-like cells could not, or only partially, be blocked with excess HLA-DR MoAB. The simultaneous addition of HLA-DQ MoAB however, could completely block the proliferation in the MLR.
In conclusion dendritic-like cells, which are superior in the MLR, require HLA-DR and/or HLA-DQ in an allogeneic MLR whereas macrophages (adherent cells) require solely HLA-DR of the MHC class II antigens.

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COMPARATIVE TUMORICIDAL ACTIVITY AND CYTOKINE SECRETION OF MACROPHAGES OBTAINED FROM DIFFERENT ANATOMICAL SITES. Viveca Sulich, Alicia V. Palleroni, Rosemary Wright, and Michael J. Brunda. Department of Oncology and Virology, Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

Macrophages from different anatomical sites were compared for their ability to become tumoricidal and to secrete interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) following stimulation in vitro by a series of biological response modifiers (BRMs). Peritoneal macrophages (PM), alveolar macrophages (AM), and tumor infiltrating macrophages (TIM), isolated from B16F10 melanoma colonies in the lung, were incubated overnight with BRMs (rMuIFN-γ, lipopolysaccharide (LPS), muramyl dipeptide (MDP)) either alone or in combination. The supernates from these cells were assayed for IL-1 and TNF-α production, while the cells were assayed for cytotoxicity against P815 target cells. PM exhibited an increased cytotoxic response following incubation with rMuIFN-γ and LPS alone but not with MDP. Secretion of IL-1 and TNF-α was observed in PM stimulated with LPS or MDP but not with rMuIFN-γ. Neither AM nor TIM were cytotoxic after incubation with any single BRM. However, both AM and TIM were induced to become tumoricidal following incubation with a combination of rMuIFN-γ plus LPS or rMuIFN-γ plus MDP. As with PM, secretion of IL-1 by AM or TIM was induced with LPS but not rMuIFN-γ. TNF-α secretion by AM was stimulated by LPS or MDP but not rMuIFN-γ ; no secretion of TNF-α by TIM was found following stimulation with any of these BRMs. These results demonstrate that different signals are needed to activate tumoricidal activity in PM versus AM or TIM, and that secretion of IL-1 or TNF-α does not correlate with cytotoxicity.

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INVESTIGATION OF THE POSSIBLE ROLE OF MACROPHAGE TISSUE TRANSGLUTAMINASE IN FC-RECEPTOR-MEDIATED FUNCTIONS. J.A. Rummage, J. Wiggins, R.W. Leu and P.A. Johnston. The S.R. Noble Foundation, Ardmore, OK 73402.

Tissue transglutaminases (TGase) are a ubiquitous group of intracellular Ca^{2+} dependent enzymes which catalyse both the covalent conjugation of polyamines to protein-bound glutamine residues and the covalent cross-linking of proteins by the formation of ε-(γ-glutamyl)-lysyl isopeptide bonds. Increased TGase

activity is associated with both the differentiation of peripheral blood monocytes into macrophages in vitro, and when elicited or activated peritoneal macrophages are compared to resident populations. Cystamine, methylamine and dansylcadaverine are substrate inhibitors of TGase which also inhibit Fc-receptor dependent phagocytosis. However, the specificity of these inhibitors is questionable and studies on receptor mediated endocytosis in cells with little or no detectable TGase activity has indicated no deficiencies in their ability to internalize Ligand-Receptor complexes. We have therefore directly compared the ability of various macrophage populations to perform Fc-receptor mediated binding, phagocytosis and antibody dependent cytolysis of sheep red blood cells to their respective levels of TGase activity. In addition we have also tested these macrophage populations for their cytolytic potential towards P815 mouse mastocytoma cells. While increased TGase activity generally correlates with increased macrophage functional competence, macrophages with low levels of TGase activity do not exhibit a proportional loss of functional activities in all cases.

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GAMMA INTERFERON ENHANCED CYTOTOXICITY BY RAT LIVER MACROPHAGES IS ASSOCIATED WITH DEPRESSED PHAGOCYTOSIS. C.R. Gardner, T.W. McCloskey, and D.L. Laskin. Rutgers University, Piscataway, NJ 08854.

Gamma interferon (g-IFN), or macrophage activating factor, is a lymphocyte derived mediator known to activate peritoneal macrophages for cytotoxicity towards tumor cells. In the present studies we examined the effects of g-IFN on liver macrophage-mediated cytotoxicity towards N1S1 hepatoma cells and phagocytosis of antibody coated sheep red blood cells (sRBC). Macrophages were isolated from livers of rats treated with 5 mg/kg lipopolysaccharide (LPS) or control by combined pronase/collagenase perfusion followed by differential centrifugation on a metrizamide gradient. Macrophages were incubated for 48 hr with increasing concentrations of g-IFN. Cytotoxicity was measured by release of ^{3}H-thymidine from prelabeled target cells. We found that g-IFN enhanced the cytotoxic activity of both resident Kupffer cells (RKC) and liver macrophages from LPS treated rats (LKC) towards N1S1 hepatoma cells. The maximum effect was observed after 72 h coincubation with an effector:target ratio of 10:1. LKC were more sensitive to the effects of g-IFN than were RKC. In contrast, g-IFN produced a dose-dependent inhibition of phagocytosis of opsonized ^{51}Cr-labeled sRBC cells by both resident and activated liver macrophages. The maximum effect was observed with 100 U/ml. Inhibition of phagocytosis by g-IFN was greater in RKC than in LKC. G-IFN treatment was also found to depress the production of hydrogen peroxide by liver macrophages. These results indicate that g-IFN enhances macrophage tumor cell killing, but depresses cellular responses associated with inflammation. Supported by NIH grant GM34310.

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INHIBITION OF BOTH ANTIBODY-DEPENDENT AND ANTIBODY-INDEPENDENT CELLULAR CYTOTOXICITY OF MOUSE MACROPHAGES BY INHIBITORS OF C1Q SECRETION. R. Leu and M. Herriott, S.R. Noble Foundation, Biomed. Div., Ardmore, OK 73402.

Complement subcomponent, C1q, is synthesized and secreted by macrophages along with other essential complement components. C1q consists of six globular heads for binding IgG and a collagen-like tail portion which binds to cellular receptors. C1q has a modulatory role in macrophage Fc and C3b receptor dependent effector functions. The collagen biosynthetic inhibitors, 3,4-dehydro-d,l-proline (DHP) and 2,2' dipyridyl (DP) have been shown to inhibit macrophage secretion of C1q which was correlated with inhibition of FcR dependent phagocytosis (Mocharla, et al., Cell. Immunol. 105:127,1987). In the present study, the effect of DHP and DP on activation of mouse peritoneal macrophages by Lipid A for antibody independent, non-specific tumor cytotoxicity was determined. Preincubation of macrophages with DHP (1-3 mM) or DP (0.1-0.3 mM) for 24 hr inhibited Lipid A dependent activation in a dose dependent fashion during a 4 hr pulse or continuous activation. Macrophages recovered from the inhibitory effects of DHP or DP after 24 hr further culture before activation. Similar pretreatment of macrophages with DHP or DP also inhibited activation by LPS, Poly I:C, and Cobra Venom Factor as well as triggering of interferon-γ primed cells by these same agents. Inhibition of ADCC activation of macrophages by DHP and DP was also reconfirmed. Our results indicate that inhibition of C1q secretion by macrophages prevents their activation by either the antibody-dependent pathways. We tentatively conclude that C1q, which binds immune complex or Lipid A and other non-antibody activators, is involved in the initiation signal for macrophage activation.

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CYTOKINE INDUCED IMMUNE ACTIVATION OF HUMAN EPIDERMAL KERATINOCYTES. T.M.Oberyszyn, R.S.Greco and F.M.Robertson, UMDNJ/Robert Wood Johnson Medical School, New Brunswick NJ 08903.

We examined the ability of soluble products from lymphocytes and macrophages to functionally activate human epidermal keratinocytes. We found that gIFN alone and in combination with TNF (100U/ml; 24-120 hrs) significantly increased the amount of class II MHC (HLA-DR) antigen on the surface of keratinocytes, while there was no significant increase with TNF alone. Using the hydrogen peroxide sensitive dye, dichlorofluorescin diacetate (DCF), and flow cytometry, we were able to identify a distinct subpopulation of keratinocytes that undergo oxidative metabolism when treated with TNF (19% over Co), gIFN (9%) and gIFN+TNF (21%). The amount of DCF oxidation was comparable to that observed in peripheral blood monocytes treated with cytokines under similar conditions. Morphological comparisons of keratinocytes and monocytes revealed similar dramatic increases in cytoplasmic vacuolation after treatment with gIFN and gIFN+TNF. Using the cloned T helper cell line, D10.G4.1, and the antigen, conalbumin, we correlated the HLA-DR expression and level of functional activation with the antigen presentation activity of cytokine-treated keratinocytes. Although gIFN treated cells expressed high levels of HLA-DR antigen, we found no increase in antigen presentation activity over that observed in untreated keratinocytes. In contrast, TNF alone and in combination with gIFN induced a significant amount of antigen presentation. This data suggests that cytokines produced by macrophages and lymphocytes are capable of inducing epidermal keratinocytes to function as activated immune cells.

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CYTOKINE MODULATION OF EPIDERMAL THYMOCYTE ACTIVATING FACTOR (ETAF)/INTERLEUKIN-1 (IL-1) PRODUCTION BY HUMAN EPIDERMAL KERATINOCYTES. F.M.Robertson, T.M.Oberyszyn and R.S.Greco, UMDNJ/Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

Human epidermal keratinocytes produce factors such as ETAF/IL-1, which serve as signals for leukocyte infiltration and lymphocyte proliferation and activation. To examine the effects of macrophage and lymphocyte products on keratinocytes, we treated cultured human epidermal keratinocytes with 100U/ml of gIFN or TNF, alone or in combination for 24-120 hours. The D10.G4.1 assay was used to

measure the levels of both soluble IL-1(sIL-1) and membrane bound IL-1(mIL-1). Our results (shown below) indicate that the mIl-1 form induced a greater amount of D10 proliferation in all of the treatment groups with the exception of TNF treated keratinocytes. gIFN and the combination of gIFN and TNF induced a significantly greater amount of both sIL-1 and mIL-1 over control levels.

	mIL-1*	sIL-1**
Control	17,773 +/- 240	10,257 +/- 2849
gIFN	65,358 +/- 1811	36,134 +/- 3310
TNF	17,841 +/- 3930	22,628 +/- 3004
gIFN+TNF	103,521+/- 3019	83,293 +/- 8234

* 1 x 10^4 keratinocytes per well.
** 1: 4 dilution of dialyzed keratinocyte supernatant.

Using the D10.G4.1 assay system, we also detected an mIL-1 inhibitory factor produced by keratinocytes at concentrations above 10^4 cells. The production of this inhibitory molecule was stimulated by gIFN as well as the combination of gIFN and TNF. The amount of inhibition observed was equivalent to a reduction in Units of IL-1 activity from 3.4 U/ml (75,680 cpm+/- 7890) to 0.8 U/ml (18,089 +/- 755) in control cultures.

These data suggest that keratinocytes produce immunomodulatory molecules that may play an important role in epidermal-leukocyte and epidermal-lymphocyte interactions.

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HEGEMONIES OF THE RETICULOENDOTHELIAL SYSTEM: BARRIER FORMING SYSTEMS OF ACTIVATED RETICULAR CELLS. L. Weiss Univ. Pennsylvania Sch. Vet. Med., Phila. PA 19104

Hegemonies are emerging in the overarching construct of the RES. The Mononuclear Phagocytic System (MPS), organized about macrophages (Mø), has been considered so fully expressive of RES activities as to render the concept of the RES outmoded. But the MPS does not include major RES cell types, as reticular cells (RC), the fibroblastic, contractile, innervated stromal cells that form the locules of splenic filtration beds. With heightened blood clearance, as in infectious disease and hemolytic anemias, I find that RC in murine and human spleen are activated (marked by signs of intense protein synthesis, dense hyaloplasm, expanded perinuclear space extended into the cytoplasm as ER, and proliferation), and augmented by immigration of circulating precursors. ARC are cytochemically different from Mø and other stromal cells by reactions for MHC antigens, alkaline phosphatase, ATP'ase, fibronectin, and collagen types. Activated RC (ARC), especially those cleared from blood, fuse and branch to become complex extensive syncitial sheets that form diverse blood-spleen barriers, ensheathe blood vessels, open or close filtration locules, trap Mø and other free cells, enclose hematopoietic colonies, and cover gaps in the circumferential reticulum limiting white pulp. In concert with the MPS, BFS thereby regulate splenic filtration and, as a consequence, hematopoiesis, immunologic activities, cell migration, blood flow and the clearance of infectious organisms and abnormal blood cells. BFS are not restricted to spleen. They occur in thymus, bone marrow, and other tissues where, as in spleen, they provide barriers. BFS, based on fibroblasts, and the MPS, based on macrophages, constitute hegemonies of the RES.

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GENETIC REGULATION OF ANTIBODY PRODUCTION TO DIFFERENT ANTIGENS IN THE MOUSE. E. Skamene, F. Gervais and D.H. Bourassa. McGill Centre for Host Resistance, Montreal General Hospital Research Institute, Montreal, Quebec, Canada H3G 1A4.

Level of humoral response to several antigens differs strikingly between A/J and C57BL/6J mice. Animals of the A/J mouse strain produce high levels of IgG antibodies to such antigens as sheep red blood cells (SRBC), bovine serum albumin (BSA) and staphylococcase nuclease (SN) while C57BL/6J mice produce low levels of antibodies to these antigens. This type of genetic control is known to be independent of immune response genes of the H-2 complex. High or low antibody production to unrelated antigens could thus be under a regulatory mechanism which might be common to all of them, such as the magnitude of antigen processing by the macrophage. In order to test this hypothesis, the level of humoral responsiveness to two unrelated antigens (SRBC,SN) was determined in AXB/BXA recombinant inbred (RI) mouse strains. This set of RI mouse strains was derived from C57BL/6J and A/J progenitors which are low and high producers of IgG to these antigens, respectively. They also exhibit numerous genetically-determined variations in macrophage function. IgG antibodies against SRBC were measured by a plaque-forming assay while IgG antibodies against SN were measured by ELISA. For each of these antigens, the level of antibody production in AXB/BXA RI mouse strains followed a pattern of continuous variation, indicating that the regulation of antibody production, was in each case, under multigenic control. Strain distribution pattern of high-, intermediate- and low response against SN and SRBC segregated independently of each other among the RI strains. Consequently, the levels of IgG antibodies against different antigens are not regulated by a common host response mechanism. We thank Dr. David Sachs (NIH) for the measurement of SN antibodies.

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PHORBOL MYRISTATE ACETATE (PMA) STIMULATED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS RELEASE CHEMOTACTIC FACTOR(S) FOR HUMAN POLYMORPHONUCLEAR LEUKOCYTES (PMNL). L.E. Odekon, M.B. Weaver, P.J. Del Vecchio, T.M. Saba and P.W. Gudewicz. Albany Medical College, Albany, NY 12208.

We have previously shown that conditioned media from PMA stimulated bovine pulmonary artery endothelial cells contains chemotactic activity for human PMNL (J. Leuk. Biol. 42:398, 1987). In the present study we extended these observations to include a human umbilical vein endothelial cell line (HUVEC, ATCC CRL 1730). Confluent monolayer cultures of HUVEC were pretreated with PMA (.010 to 0.1 ug/ml) in serum-free DMEM for 60 min and washed to remove unbound PMA. HUVEC monolayers were reincubated in DMEM containing 10% fetal calf serum and retinal derived growth factor for 18 hr at 37°C. Conditioned media from PMA treated and untreated monolayers was assayed for chemotaxis in modified Boyden chambers separated by 3 um polycarbonate filters following 30 min of incubation at 37°C. Conditioned media from PMA treated HUVEC enhanced PMNL chemotactic activity in a dose dependent manner (untreated HUVEC=13.3±1.0 PMNL/hpf, .010 ug PMA/ml=32.4±2.1 PMNL/hpf, 0.10 ug PMA /ml= 97.0± 4.0 PMNL/hpf. These data demonstrate that PMA pretreatment of human endothelial cells release soluble factor(s) that enhance PMNL migration and supports the hypothesis that the vascular endothelium is important in regulating the recruitment of circulating leukocytes to inflammatory sites. (Supported by NIH PO1 HL-32418, T32-HL-07194 and American Heart Grant #87-051G)

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PHORBOL INDUCED ADHESION OF HUMAN LYMPHOCYTES TO VASCULAR ENDOTHELIAL CELLS. L. L. Delehanty and G. M. Hebdon. Department of Chemotherapy, Glaxo Research Laboratories, Five Moore Drive, Research Triangle Park, NC 27709.

During inflammation leukocytes must traverse the vascular endothelium lining the blood vessels. Adhesion of lymphocytes to endothelium is the first step in this process. The current study was undertaken to examine the ability of lymphocytes to bind to cultured bovine pulmonary artery endothelium (CPAE). Incubation of unfractionated lymphocytes with r-interleukin-2, but not α- or γ-interferon stimulates spontaneous as well as phorbol induced adhesion to CPAE monolayers. Following phorbol 12,13-dibutyrate induced activation of protein kinase C in either lymphocytes or CPAE cells, two populations of lymphocytes bind to the endothelial monolayers: 1) a spontaneously cytotoxic population, enriched for NK cells and previously defined by their ability to nonspecifically adhere to protein surfaces and 2) a population that is noncytotoxic when used as effectors against [51CR] labeled K562 targets. The characteristics of these two lymphocyte populations will be described.

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Adherence Induction of Monocyte Mediator Genes is Regulated by Extracellular Matrices. D. Eierman, C. Johnson and S. Haskill, Depts. of Micro. and Immunol., Ob/Gyn, and Lineberger Cancer Res. Ctr., Univ. of N. Carolina, Chapel Hill, NC 27599.

Chemotaxis and monocyte adherence to endothelium and extracellular matrices during extravazation are likely to stimulate the molecular processes that determine the development of the inflammatory macrophage. We report that adherence to plastic rapidly induced TNFα mRNA while that of CSF-1 was delayed until 90 min. Steady-state levels of lysozyme mRNA were down-regulated by 4 hrs. Adherence to fibronectin (Fn) coated dishes resulted in mRNA levels approximating adherence to plastic, while adherence to Fn/anti-Fn complexes or collagen failed to induce CSF-1 or down-regulate lysozyme. In contrast, adherence to collagen induced the highest sustained levels of TNFα mRNA. With most donors, cycloheximide (CH) treatment super-induced TNFα and c-fos, had little influence on lysozyme down-regulation and had no effect on CSF-1 mRNA. However, several individuals' CSF-1 responses were inhibited by CH. We were unable to stimulate either TNFα or CSF-1 expression following exposure of non-adherent monocytes to FMLP or LPS while stimulation with PMA induced TNFα in all cases and CSF-1 in most examples. Several donors expressed high levels of CSF-1 mRNA on adherence but failed to respond to PMA under non-adherent conditions. We conclude 1. CSF-1 expression may be induced either by a PMA dependent response or one dependent upon protein synthesis. 2. preferential expression of TNFα and CSF-1 may in part be determined by the extracellular matrix. 3. chemotactic factors are incomplete activators of monocyte inflammatory mediator genes.

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THE ROLE OF MAC-1 IN ADHESION INDUCED MEMBRANE INTERLEUKIN-1 (mIL-1) EXPRESSION. M. Labadia, R.B. Faanes, and R. Rothlein (Spon: D.C. Anderson) Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877 and Baylor College of Medicine, Houston, TX 77054

The ability of peritoneal exudate macrophages (PEM) to adhere to plastic has been demonstrated to activate specific PEM functions such as tumor cell lysis and membrane Interleukin-1 (mIL-1) expression. Recently it has been reported that PEM adhesion and spreading is dependent upon the membrane glycoprotein, MAC-1. Monoclonal antibodies directed against this protein inhibit many adhesion-related functions of PEM. In this study, MAb directed at the α-subunit of MAC-1 was used to determine the role of MAC-1 in adhesion-induced mIL-1 expression on PEM's. Significant inhibition of cellular spreading and mIL-1 expression was observed when PEM's were cultured in the presence of anti MAC-1, whereas no significant inhibition of either parameter was seen with control antibody directed against Class I antigen. To ensure that anti-MAC-1 did not inactivate the PEM's ability to generate mIL-1, PEM were exposed to soluble stimuli such as LPS in the presence of anti MAC-1. The data indicates that anti-MAC-1 had little or no effect on LPS (1 μg/ml) induced mIL-1 expression. This observation indicates that the cells are capable of producing mIL-1 in the presence of anti-MAC-1, implying that the inhibition seen using anti-MAC-1 on adhesion induced mIL-1 expression was due to interference with PEM's adherence rather than a nonspecific metabolic disturbance or membrane perturbations caused by the antibody. When EDTA, a known inhibitor of MAC-1 function, was added to cultures, inhibition of cellular spreading and mIL-1 expression of PEM's was noted. However, when PEM's were co-cultured with EDTA and LPS, no inhibition of mIL-1 expression was observed. These results suggest that MAC-1 does not play a significant role in LPS mediated expression of mIL-1. Additionally, adhesion and LPS induce mIL-1 by independent means and that MAC-1 plays a role, only in adhesion related mIL-1 expression.

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FLOW CYTOMETRY CHARACTERIZATION OF MURINE MICROGLIAL CELLS MAINTAINED IN IN VITRO CULTURE. N. Hassan, J. Rothmann, D. Campbell, S. Rifat and S. Douglas. Div. Allergy-Immunology-BMT, Children's Hosp. of Phila., Univ. of PA Medical School, Phila., PA 19104.

Monocytes-macrophages express different surface receptors for FcR, C3bi, MHC Class II, CD4 and fibronectin. Brain macrophages or microglia have been studied only to a limited extent. Microglia were first described by Rio-Hortega (1932) using a weak silver carbonate stain. The concept that brain microglia are morphologically and functionally distinct cell subpopulations is controversial. Mononuclear phagocytes of the brain have been considered as the effector cells in the immunopathogenesis of multiple sclerosis, experimental allergic encephalomyelitis and acquired immunodeficiency syndrome. In this study we investigated the expression of surface receptors on isolated murine microglia maintained in in vitro cultures. Newborn mouse (BALB-C) brains were dissected, minced and cells maintained in in vitro culture for 2 weeks. Microglia with vacuolated cytoplasm and extended short processes were observed on top of a monolayer of astrocytes. Cells were shaken for 16 hrs at 200rpm, 37°C, collected, and stained for different markers of macrophages and glial cells. Specific immunofluorescence were analyzed using an EPICS C flow cytometer and percent positive cells were determined

following its subtraction from the control (isotype matched immunoglobulins).

EXP	Mac1	Mac3	F4/80	Ia	L3T4	GFAP	GALC	Fibronectin
1	56%	-	46%	0%	-	-	-	-
2	70%	-	-	-	0%	0%	0%	6%
3	72%	13%	-	-	-	0%	0%	10%
4	61%	21%	47%	0%	9%	-	-	-

We conclude that the microglial cell purity by the shaking method is approximately 64% (by Mac1) and that the expression of the different macrophage markers on the cells is heterogeneous. Astrocytes and oligodendrocytes are not detected and the microglia do not express the MHC Class II antigen. A small percentage express the L3/T4 epitope. (Supported in part by grants from NIH HL-27068, NS-17752, AmFar 161, and MS Society 1919A-1).

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ABILITY OF INTERLEUKIN-1 TO MINIMIZE CYCLOPHOSPHAMIDE INDUCED HEMATOPOIETIC TOXICITY: EVIDENCE FOR AN EFFECT MEDIATED BY STROMAL CELLS. V.S. Gallicchio, M.J. Messino, B.C. Huelette, T.A. Kar-Mirza, D. Friedman, and M.A. Doukas. Hematology/Oncology Division, University of Kentucky Medical Center, Lexington, KY, 40536.

The suppression of hematopoiesis is the major limiting toxic side-effect of many chemo-radiotherapy regimens for malignant disease. The macrophage derived molecule, IL-1, is a cytokine with multifunctional properties related to normal hematopoiesis, such as the potentiation of colony stimulating factor production (CSF). IL-1 has been implicated as an effective agent in minimizing the cytotoxicity associated with drugs and/or radiation by its ability to reduce myelosuppression. To further investigate the use of IL-1 in ameliorating drug-induced toxicity, C3H/HeJ female mice were pre-treated with either 500 or 1000 LAF units of recombinant human IL-1 α, i.p. (Hoffmann-LaRoche). Within 2-4 hrs following rhIL-1, mice received a single injection of cyclophosphamide (CTX)(200 mg/kg bw, i.v.). Beginning 24 hrs later, groups of mice were serially sacrificed for assessment of their hematological and stem cell parameters. Bone marrow and spleen were evaluated for CFU-E, BFU-E, CFU-GM, CFU-Meg, CFU-Mix, and stromal progenitors. CTX alone produced significant suppression of all stem cells whether of marrow or splenic origin. Mice pre-treated with rhIL-1 before CTX demonstrated enhanced recovery of all stem cells; however what was significant was that stromal colonies were not significantly reduced in animals given IL-1 before CTX and in fact were equal to PBS controls or greater than the CTX controls; e.g., day 1 BM IL-1/CTX were 192% of CTX controls; day 4-253%, day 14-1005%. These results indicate IL-1 ameliorates the myelotoxicity of cancer therapy by protecting the hematopoietic microenvironment from the toxicity induced by either drugs and/or radiation. (Supported by grants CA-33652 and CA-45259 from the National Cancer Institute).

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SYNTHESIS OF INTERLEUKIN-1 (IL-1) BY HUMAN MONOCYTES CULTURED IN VITRO WITH AMPHOTERICIN B (AmB). D.L. Hoover, J.B. McClain, A.S. Dobek, T.A. Olson, C.A. Nacy, and B. Joshi. Walter Reed Army Medical Center and Walter Reed Army Institute of Research, Washington, DC 20307.

Fever and chills in patients treated with AmB may be mediated by monocyte-derived prostaglandins (PG): AmB induces release of PG in vitro, and symptoms can be prevented by treatment with inhibitors of PG synthesis in vivo. To determine whether AmB also induces production of IL-1, a potent stimulus for PG release, we cultured one million plastic-adherent mononuclear cells from normal volunteers for 20 hr in dilutions of AmB or lipopolysaccharide (LPS) in RPMI. IL-1 content of supernatant fluids was assessed in a comitogenesis assay using D10 cells. Cells cultured with up to 2.5 ug/ml AmB or 10 ug/ml LPS released as much as 200 U IL-1 in a dose-related manner. IL-1 activity was neutralized by treatment with anti-IL-1 beta. AmB did not simply cause release of intracellular IL-1 induced by adherence to plastic: lysates of cells cultured with RPMI, AmB, or LPS contained 6, 70, and 70 U IL-1, respectively. Corresponding supernatant fluids contained 2, 70 and 200 U IL-1. AmB may induce IL-1 synthesis differently than LPS: cotreatment of cells with AmB and gamma interferon (IFN) minimally enhanced AmB-induced IL-1 release. In contrast, IFN enhanced IL-1 production by LPS-treated monocytes at least 10-fold. These studies document IL-1 production by AmB-stimulated human monocytes. In addition, they suggest that AmB may induce monocytes to synthesize or release IL-1 by a mechanism distinct from that of LPS.

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INTERLEUKIN 1: A GROWTH FACTOR AND INDUCER OF DIFFERENTIATION FOR K-562 CELLS. A.T. Ichiki, W.D. Edmondson, J.T. Crossno, Jr., D.A. Gerard, D.A. Suganthraj, E.G. Bamberger, C.B. Lozzio. Univ. TN. Med. Ctr./Knoxville, Knoxville, TN 37920

The K-562 cell line is a pluripotent leukemia line which can differentiate along the erythrocytic, megakarycytic, myelocytic, and lymphocytic lineages following appropriate inducers of differentiation. We have been probing the effects of lymphokines and cytokines on K-562 cells as inducers of differentiation or as growth factors. Interferon-γ resulted in the expression of HLA class I antigens. The cells express the receptor for interleukin ? (IL2) and are affected by IL2 treatment as detected by the expression of the CD4 antigen. In this study, we probed the effects of purified IL1 (pIL1) and recombinant IL1β (rIL1β). The proliferation of K-562 cells treated with pIL1 (8 u/ml) was greatly enhanced 24 and 48 hr later and, to a lesser degree, at 2 u/ml as compared to untreated cells. This enhanced proliferation resulted in decreased DNA synthesis 72 and 96 hr later. Hence, pIL1 appears to function as a growth factor for K-562 cells. Light microscopy indicated that pIL1-treatment resulted in considerable vacuole formation and a reduced nuclei to cytoplasm ratio. There was also an increased number of cells with lobulated nuclei, suggesting that the cells had undergone a differentiation process. At a concentration of 10 u/ml, rIL1β enhanced DNA synthesis 24 hr later, while increased proliferation was detected with 2.5 u/ml 96 hr later. There appears to be a difference in the effects of the levels of added rIL1β. Higher levels caused an early effect while lower levels resulted in a later effect. The light microscopic observations with rIL1β-treated cells were not as dramatic as with pIL1-treated cells. Electron microscopic studies did demonstrate a detectable increase in nuclear lobulation as well as an increase in Golgi and rough endoplasmic reticulum profiles with the rIL1β-treated cells. The EM observations are consistent with an increase in cellular differentiation of K-562 cells. pIL1 and rIL1β are both a growth factor and inducer of differentiation for K-562 cells.

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COMPARISON OF IN VIVO EFFECTS OF HUMAN RECOMBINANT IL 1 AND IL 6 IN RADIOPROTECTION AND INDUCTION OF EARLY AND LATE ACUTE PHASE REACTANTS. R. Neta, S.N. Vogel, G.G. Wong, and R.P. Nordan. AFRRI, USUHS, NIH. Bethesda, MD, and GI, Boston, MA.

In view of recent findings that IL 1 and IL 6 share a number of biological activities, including induction of fever, neutrophilia and acute phase response, and that IL 1 induces IL 6 in fibroblasts and macrophages, it was proposed that IL 6 mediates many IL 1 activities. To test this hypothesis in vivo we assessed induction of IL 6 following IL 1 administration to mice as well as tested IL 6 for radioprotection and induction of early (CSF) and late (fibrinogen) acute phase response. IL 1 given to mice ip in a dose of 1000

ng induced IL 6 in circulation with maximal titers at 2 hrs. However, unlike IL 1 which is radioprotective when administered in doses above 100 ng/mouse, doses of 100-3000 ng/mouse of IL 6 did not result in increased survival of mice following lethal irradiation. In fact, such treatment given 20 hrs before LD50/30 doses of radiation resulted in reduced survival of mice. Whereas IL 1 in doses above 10 ng/mouse induced at 2 to 6 hrs a dose dependent increase in CSF in circulation, IL 6 did not induce detectable levels of CSF at 2, 6 and 20 hrs after administration. Injection of IL 6 to mice produced a dose dependent increase in circulating fibrinogen, with 100 ng inducing 140+20% and 2000 ng = 175+9% of control. However, similar administration of IL 1 resulted in much greater increase in fibrinogen with 100 ng producing an increase of 240+15% and 2000 ng 380+11%. Therefore, IL 1 is a more effective inducer of fibrinogen in mice than is IL 6. Although conclusive results as to the relative roles for IL 1 and IL 6 in vivo will await availability of anti IL 1 and anti IL 6 antibodies our data at present do not support the suggestion that the above IL 1 effects can be attributed to IL 6.

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HUMAN TONSILLAR LYMPHOCYTES RELEASE LYMPHOKINES THAT ALTER HUMAN IN VITRO LYMPHOCYTE MIGRATION. R.G. McFadden, K. Vickers, L.J. Fraher (Spon: P. Lala) Lawson Research Institute and University of Western Ontario, London, Canada, N6A 4V2.

The majority of lymphocytes present at sites of delayed-type hypersensitivity reactions (DTHR) are not specifically sensitized for the antigen eliciting the reaction. Human lymphokines have been described that alter the migration of unsensitized lymphocytes. Methods: We cultured human tonsillar nylon wool-nonadherent mononuclear cells with either concanavalin A (1 μg/ml) or histamine (100 μM). The dialyzed supernatants were directly pumped onto a Novapak C18 HPLC column and eluted using an acetonitrile gradient. Collected fractions were assayed for chemokinetic activity using modified Boyden microchemotaxis chambers; 8 μm filters separated test substance from human peripheral blood mononuclear cells (PBMC). Results: Five discrete peaks of chemoattractant activity were identified, as well as one negative chemokinetic factor. Further HPLC fractionation and in vitro migration assays showed that the lymphocyte chemoattractant factors (LCF) did not represent the effects of interleukin-2, tumor necrosis factor-α, or gamma interferon. LCF-IV eluted from the HPLC column in the approximate position of interleukin-1, and its chemokinetic effect was blocked by anti-IL-1 antibody. The previously described LCF derived from human PBMC was found to be heterogenous on HPLC fractionation, corresponding to LCF peaks I and III. Conclusion: Human tonsillar lymphocytes secrete novel mediators that can alter the migration of unsensitized human lymphocytes; these lymphokines may play an important role in the accumulation of these cells at sites of DTHR. (Supported by MRC and NIN Canada)

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EFFECT OF CYTOKINES ON POLYMORPHONUCLEAR NEUTROPHIL (PMN) INFILTRATION IN THE MOUSE: INDUCTION OF INFILTRATION BY INTERLEUKIN 1 AND TUMOR NECROSIS FACTOR. T.A. Wiltrout,[1] A. Pilaro,[2] and T. Sayers[1] (Spon: R. Wiltrout). [1]BCDP, Program Resources, Inc. and [2]Laboratory of Experimental Immunology, BRMP, NCI-FCRF, Frederick, MD 21701-1013.

Cytokines were injected into the peritoneal cavity of BALB/c mice. Cytokines included interleukin 1α, interleukin 1β (IL1α, IL1β), tumor necrosis factorα, tumor necrosis factorβ (TNFα, TNFβ), interferonα (IFNα), interferonγ (IFNγ), interleukin 2 (IL2), interleukin 3 (IL3), granulocyte macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF). All cytokines were injected at low protein concentrations (a maximum of 50 ng per mouse) and dose responses were performed in all cases. Peritoneal leukocytes were isolated at various time points, cytocentrifuge preparations were made and cells were fixed, stained and counted microscopically. The injection of low doses of IL1α or IL1β (5-0.005 ng per mouse) resulted in a very large influx of PMN into the peritoneal cavity by 2 hrs after injection with optimal infiltration occurring with 0.5 ng IL1. Of the other cytokines tested, only TNFα or TNFβ (lymphotoxin) were able to induce a significant infiltration of PMN. However, based on protein administered, about 100 times more TNF than IL1 was required to produce comparable infiltration. Combination of suboptimal amounts of TNFα and IL1 resulted in a synergistic enhancement of peritoneal PMN infiltration. In all cases no PMN infiltration was detectable 30 min after injection, however slight increases were noted at 1 hour. The response peaked at 2 hours was maintained up to 12 hours and declined somewhat by 24 hours. Neither IL1 nor TNF were chemotactic for PMN when assayed in vitro using Boyden chambers. These data suggest that local production of inflammatory cytokines like IL1 or TNF can induce extensive PMN infiltration. These cytokines may induce the production of endogenous chemotactic factors, or act on other stages of PMN emigration.

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REQUIREMENT OF LIPID A-ASSOCIATED PROTEINS (LAP) BY rIFN-γ PRIMED C3H/HeJ (Lps^d) MACROPHAGES (Mϕ) FOR TNF PRODUCTION. M. Michele Hogan and Stefanie N. Vogel. U.S.U.H.S., Bethesda, MD 20814.

It has been shown that activation of murine Mϕ to a fully tumoricidal state requires specific signals be delivered in a step-wise manner: a "priming" signal first renders the Mϕ responsive to a "trigger" signal. One potent "priming" signal is IFN-γ and one often used "trigger" signal for endotoxin-responsive (Lps^n) C3H/OuJ Mϕ is lipopolysaccharide (LPS). In contrast, rIFN-γ-primed C3H/HeJ Mϕ can not become tumoricidal in response to protein-free phenol-water extracted LPS (PW-LPS), but become tumoricidal when exposed to protein-rich butanol-extracted LPS (But-LPS) or purified LAP. Further studies showed that in vitro production of TNF also required two signals; rIFN-γ and a second signal that contained LAP. rIFN-γ-primed C3H/HeJ Mϕ failed to produce TNF in response to any concentration of PW-LPS. Failure to detect TNF was not due to the production of low quantities of a 16X concentration of supernatants also failed to show any functional TNF activity. While exposure of rIFN-γ-primed C3H/HeJ Mϕ to LAP resulted in a fully tumoricidal state equivalent to C3H/OuJ Mϕ, the levels of TNF produced remained discrepant. Under identical conditions, C3H/OuJ Mϕ produced approximately 5-fold more TNF (11,776 U/ml) than C3H/HeJ Mϕ (2,399 U/ml). This suggests that although C3H/HeJ Mϕ can respond functionally in a "normal" manner given the correct signals, they remain quantitatively deficient in the production of certain proteins. TNF production and Mϕ-mediated tumor cell lysis were shown to be dissociable events. The tumor target used in these studies (P815) was shown to be resistant to as much as 40,000 U/ml purified rTNF and failed to be lysed by C3H/OuJ Mϕ which produced high levels of TNF after treatment with only LPS. Also, P815 was resistant to combination of purified rIL 1, rTNF and rIFN-γ. Lastly, rabbit anti-mouse TNF antibody added to stimulated Mϕ cultures at a concentration known to abrogate TNF activity in a standard TNF assay, had no effect on the induction of tumor cell lysis. (NIH Grant # AI-18797)

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TUMOR NECROSIS FACTOR (TNF) AND INTERLEUKIN-1 (IL-1β) mRNA HALF-LIVES ARE REGULATED BY A SHORT-LIVED RNase. J. Economou, R.Essner, K.Rhoades, W.McBride, D.L.Morton. Division of Surgical Oncology, Department of Radiation Oncology, UCLA Medical Center, Los Angeles, CA 90024.

HL-60 cells can be induced to produce TNF and IL-1 mRNA by phorbol myristate acetate (PMA, 1μg/ml). The protein synthesis inhibitor cycloheximide (CY,10μg/ml) alone or in combination with PMA will induce markedly increased amounts of mRNA. Cells were cultured for 4 hr with various combinations of PMA and CY. Whole cellular RNA was extracted by the urea lysis method and run on glyoxal gels and transferred to nylon membranes. Blots were hybridized with ^{32}P-labelled cDNA probes for TNF and IL-1 . Representative densitometric scans from these Northern analyses were: no addition - 1.58, PMA-47.2, CY-44.2, PMA+CY - 80.7. TNF and IL-1 mRNA migrated in the region of the 8S rRNA band. The higher level of mRNA could be due to increased transcription or longer mRNA t½. HL-60 cells were activated with PMA or PMA+CY for 4 hr at which time actinomycin D(10μg/ml) was added to the culture. RNA was isolated at 0, 1 and 2 hr after addition of actinomycin D and analyzed by Northern hybridization. By 2hr, mRNA in the PMA group was barely detectable whereas nearly the same amount was detectable at 0, 1 and 2 hr in the PMA+CY group. These results indicate that inhibition of de novo protein synthesis results in increased levels of TNF mRNA due, in part, to a lengthening of the mRNA t½. Message degradation is probably mediated by a short-lived RNase.

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AGE AND SENESCENCE; ROLE OF CACHECTIN/TUMOR NECROSIS FACTOR (TNF). SF Bradley, SL Kunkel, and CA Kauffman. VAMC and U of Michigan, Ann Arbor, MI 48105.

It has been suggested that senescence and the cachexia of aging may be mediated in part by TNF. This study assessed the effects of aging and moderate protein malnutrition, a frequent concomitant of aging, on TNF production. Fischer rats (3 mo, 12 mo, and 24 mo) were fed either a standard or a low protein diet for 6 weeks. Peritoneal macrophages were elicited with shellfish glycogen given intraperitoneally 3 days prior to the experiment. The macrophages were harvested, washed, and stimulated with killed Staphylococcus epidermidis in the presence of 10% human serum. The supernatants were harvested and were assayed in an L929 fibroblast lytic assay for TNF activity.

	Standard Diet		Low protein diet	
age (mo)	N	TNF (u/ml)	N	TNF (u/ml)
3	15	507 ± 23	15	432 ± 43
12	13	229 ± 29	13	300 ± 40
24	12	296 ± 36	16	211 ± 35

No significant differences in TNF activity were noted in the macrophage supernatants from rats of 3 different ages; moderate protein malnutrition did not decrease TNF secretion. Increasing age and moderate protein malnutrition do not appear to influence TNF secretion by peritoneal macrophages of Fischer rats.

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MODULATION OF ARACHIDONIC ACID METABOLISM BY BOVINE ALVEOLAR MACROPHAGES EXPOSED TO INTERFERONS. M.G. O'Sullivan, N.J. MacLachlan, L.N. Fleischer, N.C. Olson, and T.T. Brown, Jr., College of Veterinary Medicine, North Carolina State University, Raleigh, N.C. 27606

As part of studies on inflammatory lung disease, the in vitro production of arachidonic acid (AA) metabolites by alveolar macrophages (AM) was investigated. Bovine AM were cultured in 6 or 12 well plates at 2 or 1 x 10^6/well respectively. After overnight incubation, AM ($2x10^6$/well) were washed and stimulated with A23187 or opsonized zymosan (OPZ) for 5, 10, or 40 minutes and 0.5, 1, or 2 hours respectively. These stimuli caused time dependent increases in LTB_4, TXB_2, and $PGF_{2\alpha}$ as measured by radioimmunoassay of culture supernatants. Largest amounts were present after 40 minutes for A23187 and 2 hours for OPZ.

	A23187	Control	OPZ	Control
LTB_4(pg/0.1ml)	680±232*	<6	19±16	<6
TXB_2(pg/0.1ml)	138±50*	9±6	143±59*	8±2
$PGF_{2\alpha}$(pg/0.1ml)	36±17*	5±1	30±11*	<5

(p <0.05 = *) (n = 3)

The effect of interferons (IFN) was studied by culturing AM ($1x10^6$/well) overnight with recombinant bovine IFN-γ (10^3 units), IFN-α (10^6 units) or medium alone and subsequently stimulating with A23187 or OPZ.

Stimulus	A23187 (n=6)			OPZ (n=6-8)		
Culture condition	Medium	IFN-α	IFN-γ	Medium	IFN-α	IFN-γ
LTB_4(pg/0.1ml)	224±44	331±55*	381±68*	7±2	18±6	19±6
TXB_2(pg/0.1ml)	13±5	21±4	193±57*	46±16	29±6	521±60*
$PGF_{2\alpha}$(pg/0.1ml)	<5	<5	43±11*	13±6	7±2	153±27*

These results indicate that IFN-γ primes AM for a marked increase in cyclooxygenase products whereas both IFN-γ and -α prime for a modest increase in LTB_4 production upon subsequent stimulation. OPZ is an effective stimulus for PG but not LT production whereas A23187 is a potent stimulus for LT production. The priming effect of IFNs on AA metabolism by AM may play a role in the development of pulmonary inflammation; for example, in the development of bacterial pneumonia secondary to viral infections associated with IFN production.

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REDUCTION OF COLLAGEN BIOSYNTHESIS OF VASCULAR ENDOTHELIAL CELLS BY MONOKINES IN VITRO

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In lung tissue the endothelium is involved in multiple metabolic and detoxification functions, and, moreover, it contributes to blood flow regulation. Irritation of pulmonary endothelium may result in a remarkable destortion of the funtional properties of the lung. Vascular endothelial cells in situ and in vitro undergo morphological alterations at sites of cell-mediated or humoral immune responses. In the present in vitro study, macrophage conditioned medium was used for "activation" of endothelial cells derived from human umbilical cords. The endothelial cells have been incubated with ^{14}C-glycin for 24 hours for biochemical analysis of the total protein and collagen biosynthesis. The ^{14}C-proteins were characterized by gel electrophoresis and by encymatic digestion either with pepsin or collagenase. The results showed that non-activated endothelial cells synthesized collagen types I, III and V, and to some extent collagen type IV. However, obviously monokines reduced the total protein synthesis and the collagen biosynthesis. The results showed that the regulation of collagen biosynthesis of endothelial cells can be mediated by macrophages.

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CHARACTERIZATION OF A MONOCLONAL ANTIBODY AGAINST A RECEPTOR PROTEIN FOR MOUSE GAMMA INTERFERON. M. Basu, J.L. Pace, D.M. Pinson and S.W. Russell. Univ. Kansas Medical Center, Kansas City, KS 66103.

Brown Norway rats were immunized with mouse gamma interferon receptor preparations which had been solubilized from WEHI-3 plasma membranes with 1% Triton X-100 and enriched by ligand affinity chromatography. Spleen cells from an immunized rat were fused with mouse Sp2/0-Ag14 myeloma cells. Thirty hybridomas (out of 1,500 screened) were found to produce antibodies that inhibited specific binding of MuIFN-γ to WEHI-3 cells. These were selected and cloned by limiting dilution. One of these, GR-20, produced an IgG2a antibody that completely inhibited the binding of ^{125}I-MuIFN-γ to WEHI-3 cells. This antibody also bound to the surfaces of cultured bone-marrow derived macrophages as detected by a whole cell ELISA. The GR-20 antibody did not react with MuIFN-γ as determined by radioimmunoassay. When coupled to Sepharose beads, it removed > 90% of the specific binding activity for MuIFN-γ that was present in membrane lysates of WEHI-3 cells and immunoprecipitated a 95 kDa protein from the cell lysates of ^{125}I surface labeled WEHI-3 cells. In addition, pretreatment of macrophages with GR-20 antibody prevented the development of tumoricidal activity when these cells were stimulated with MuIFN-γ and LPS. Also, GR-20 antibody inhibited the antiviral activity of MuIFN-γ in mouse L-cells. Taken together these studies suggest that the GR-20 antibody recognizes an epitope on the MuIFN-γ receptor that is important for functional activity. Supported by NIH grants CA38779 and RR00037.

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MACROPHAGE FUCOGANGLIOSIDES EXIST AS THREE SEPARATE SPECIES. C.S. Berenson, H.C. Yohe and J.L. Ryan. VAMC and Yale Univ. School of Med., West Haven, CT. 06516.

Gangliosides are amphipathic, sialic acid-containing glycolipids concentrated in the plasma membranes of eukaryotic cells. They have been implicated as receptors for a wide variety of ligands including bacterial toxins and immunoregulatory cytokines. Macrophage gangliosides have been shown to increase in complexity in response to LPS, and may have a down-regulatory role in LPS-induced lymphocyte mitogenesis. Fucogangliosides hold particular key roles in cell interactions. They have been noted to increase or appear de novo in cells undergoing malignant transformation. Collective observations have implicated macrophage fucogangliosides as the receptor for macrophage migration inhibitory factor (MIF). A particular obstacle to defining an isolated macrophage fucoganglioside as the MIF receptor is posed by the difficulty of separation. Thioglycollate-elicited peritoneal macrophages contain over 30 distinct gangliosides on two-dimensional thin layer chromatography (TLC). To investigate the composition of macrophage fucogangliosides, thioglycollate-elicited murine peritoneal macrophages were incubated with ^{3}H-fucose (2 uCi/10^{6} cells) in vitro. Optimal labeling was achieved in 48 hr. Macrophage gangliosides were purified by a series of chromatographic separations. Analysis by 2D autoradiography demonstrated the presence of three distinct fucogangliosides, all located in the more heavily-sialated ganglioside region. Scintillation counting of small regions of radiolabeled gangliosides, removed from 2D TLC plates by scraping, confirmed the presence of fucogangliosides in these regions. We are currently isolating the fucogangliosides using a two-solvent HPLC gradient processing system, with a silica-amine preparative column. We have successfully separated the complex ganglioside mixture of thioglycollate-elicted macrophage gangliosides into fractions each containing one to three individual gangliosides. Purity has been confirmed by TLC. The presence of at least three fucogangliosides in the macrophage cell membrane raises the possibility that MIF receptor function could reside in more than one membrane component. In order to determine if any or all of these fucogangliosides function as the MIF receptor, we are currently purifying each, by HPLC, for analysis of MIF receptor function.

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ADMINISTRATION OF PURIFIED MONOCLONAL ANTIBODY TO L3T4 IMPAIRS THE RESISTANCE OF MICE TO LISTERIA MONOCYTOGENES INFECTION. C. Czuprynski, J. Brown, K. Young, and J. Cooley. Univ. WI School Vet. Med., Madison, WI 53706.

Our existing understanding of the contributions of T cell subsets to anti-listeria resistance is based principally on adoptive transfer experiments that utilized T cells obtained from immunized donors, or listeria-specific T cell lines. In this study we attempted to determine the in situ contributions of L3T4^{+} cells to anti-listeria resistance. Mice received 0.2 mg purified anti-L3T4 mAb (GK 1.5) i.p. on three consecutive days, followed 24 hours later by a sublethal L. monocytogenes challenge. Anti-L3T4 mAb treatment retarded, but did not completely eliminate, clearance of listeriae from the spleen and liver as compared to mice treated with an irrelevant mAb of the same isotype. Concomitant with this decreased clearance we observed a profound depression of T cell responsiveness to listeria antigens. Anti-L3T4 mAb treated listeria-infected mice failed to exhibit delayed type hypersensitivity when injected with soluble listeria antigens, whereas control listeria-infected mice developed a vigorous delayed type inflammatory response. In addition, spleen cells obtained from anti-L3T4 mAb treated listeria-infected mice proliferated poorly and failed to produce detectable IFN-γ when stimulated with listeria antigens and mitogens in vitro. Serum CSA levels, on the other hand, were elevated to a similar extent in anti-L3T4 mAb treated and control mice during the course of a primary L. monocytogenes infection. Anti-L3T4 mAb treated listeria-immunized mice exhibited some elevation of resistance to rechallenge with L. monocytogenes. In addition, their spleen cells transferred a modest level of protection to naive recipients. Both responses were reduced, however, as compared to control listeria-immunized mice. These results indicate that L3T4^{+} cells are required for optimal expression of anti-listeria resistance, however, compensatory defense mechanisms generate substantial resistance even when there is profound impairment of certain T cell functions.

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THE C5-SUFFICIENT A/J CONGENIC MOUSE STRAIN: INFLAMMATORY RESPONSE AND RESISTANCE TO *LISTERIA MONOCYTOGENES*. F. Gervais, C. Desforges and E. Skamene
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A/J mouse strain poorly responds to an inflammatory stimulus and is highly susceptible to *Listeria monocytogenes* (Lm) infection. This defect in the phagocyte inflammatory response caused by the C5 component of complement deficiency was shown, by linkage analysis, to be the major reason for the extreme susceptibility of A/J mice to Lm infection. The importance of this genetic defect in C5 in relation to the poor macrophage inflammatory response and to the susceptibility to Lm infection was evaluated by developping a C5-sufficient congenic A/J mouse strain. This A/J.C5 mouse strain was studied for its inflammatory response and for its susceptibility to Lm infection. C5-sufficient congenic A/J.C5 mice showed a slight improvement (2x) in their level of macrophage inflammatory response, however, they did not mount a response as strong as the Listeria-resistant C57BL/6J which donated the C5 allele. When infected with Lm, A/J.C5 mice were found to be as resistant as C57BL/6J mice. These results suggest that the presence of C5 on an A/J background partially improves the deficient macrophage inflammatory response. This increase is sufficient to render the A/J.C5 mouse strain highly resistant to Listeria infection. A/J.C5 mouse strain represents a new tool for the study of the importance of C5 in resistance to infection and in the regulation of the macrophage inflammatory response.

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INTERFERON GAMMA ENHANCES HERPES SIMPLEX TYPE 1 REPLICATION IN HUMAN MONOCYTES:

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Studies to explore the role of human mononuclear phagocytes and Interferon in Herpes Simplex Type 1 (HSV-1) infection were conducted. Freshly isolated human monocytes (obtained from leukapheresis and counter-current flow elutriation) exposed to HSV-1 were abortively infected as determined by viral antigen expression but lack of infectious virus production. When monocytes were cultured several days prior to infection, they became highly susceptible to a productive lytic infection. Cell lysis was prevented following pre-treatment with 100IU/ml of Interferon alpha; however, Interferon gamma at similar or higher doses was not effective. When freshly isolated monocytes were treated with alpha or gamma Interferon, "activation" was established, as defined by induction of HLA-Dr antigens, Hydrogen Peroxide release and tumoricidal activity. Paradoxically, Interferon gamma "activated" cells were highly susceptible to HSV-1, as manifested by cell associated viral antigen expression, cell fusion, release of infectious virus and cell lysis. Treatment of monocytes with Interferon alpha did not result in enhanced susceptibility to HSV-1 infection.

This observation raises an important question: Does monocyte "activation", as defined by the above criteria, result in the stimulation of cellular mechanisms which enhance Herpes virus replication?

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PHORBOL-INDUCED MONONUCLEAR PHAGOCYTE DIFFERENTIATION ALTERS PERMISSIVENESS TO INFLUENZA A INFECTION.

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Mononuclear phagocytes play an important role in host defense against viral infection; their ability to control the extent of virus replication and spread is thought to be dependent on the stage of differentiation. Using a human mononuclear phagocyte cell line HL60 we have characterized the effect of differentiation from promyelocytic to monocytoid state (defined by the established criteria of morphology and alpha-naphthyl esterase expression) on permissiveness to influenza A virus. HL60 cells were induced to differentiate to monocytoid state by exposure to the phorbol ester phorbol 12-myristate 13-acetate(TPA) which is known to activate protein kinase C, a limb of the inositol-lipid second messenger system. The yields of infectious virus from HL60 cultures were compared with the permissive epithelial cell line MDCK. Cells in the promyelocytic stage produced $5x10^5$PFU/10^6cells at 24 hrs post infection (PI). Differentiation to a monocytoid state by exposure to 1 μM TPA for 36 hrs prior to infection was accompanied by a 15-fold decrease in infectious virus production, $8.2x10^3$PFU/10^6 cells at 24 hrs PI. Exposure of HL60 cells to TPA for 30 min before infection did not alter the yield of infectious virus produced at 24 hrs PI, $4.9x10^5$PFU/10^6cells. Incubation of MDCK cells with TPA for 36 hrs did not alter influenza A virus yield, $3.5x10^6$ vs $7.3x10^6$PFU/10^6cells. Hemadsorption and an indirect assay using horseradish peroxidase conjugated secondary antibody and polyclonal antisera to influenza A demonstrated that approximately 50% of the cells in all cultures were infected,as determined by viral protein expression.SDS-PAGE and autoradiographic analysis of ^{35}S-methionine/cysteine labelled cell lysates showed synthesis of the major viral polypeptides in both differentiated and control HL60 cells. These data suggest that the decrease in permissiveness to influenza A virus is a characteristic acquired during TPA induced differentiation of HL60 cells. As we have previously shown for primary mononuclear phagocytes, differentiated HL60 cells synthesize viral proteins but do not produce infectious virus, suggesting a block at a later stage of viral replication.

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HIV PRODUCTION BY CULTURED MACROPHAGES CAN BE REGULATED BY INTERFERON (IFN), CYTOKINES, AND BACTERIAL LIPOPOLYSACCHARIDE (LPS).

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Agents which affect macrophages were examined for their effects upon the ability of macrophages to serve as host cells for HIV replication. Monocytes were isolated from the blood of seronegative volunteers by adherence to fibronectin and cultured in low endotoxin media for 5 days. The adherent cells were infected with HTLV-III/Ba-L85, a macrophage-tropic form of HIV-1 (the gift of Dr. M. Popovic, NCI), and virus production was monitored using an ELISA for p24 core antigen. The presence of multinucleated giant cells was used as an indication of cytopathology. Under these conditions, rIFN-γ, natural IFN-α, and LPS markedly reduced virion release from infected macrophages and prevented the formation of multinucleated giant cells. In contrast, granulocyte-macrophage CSF (GM-CSF) variably enhanced virion release and cytopathology. IL-1, IL-2, IL-4, and TNF-α were without significant effects at the doses tested. IFN-γ, IFN-α, and LPS were active even when added one day after viral infection, suggesting that these agents do not affect the early events of virus binding, penetration, and uncoating. In dose response studies, less than 100 U/ml of IFN-γ or 1,000 U/ml of IFN-α maximally prevent virion release and completely prevented cytopathology. These data indicate that HIV production by macrophages *in vitro* may be regulated by an inducible host cell mechanism. These findings may explain the restricted replication of HIV in macrophages *in vivo* and suggest an antiviral role for interferons in the therapy of HIV infection.

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INVESTIGATION OF THE BLOCK IN VIRAL mRNA AND PROTEIN SYNTHESIS IN INTRINSIC RESISTANCE OF MOUSE RESIDENT PERITONEAL MACROPHAGES (PMØ) AND KUPFFER CELLS (KC) TO HERPES SIMPLEX VIRUS TYPE 1 (HSV-1)

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Using in vitro single cycle infections with HSV-1, strain KOS, we have recently demonstrated (J. Gen. Virol. 66:1123, 1985;1988) that MØ show heterogeneity in resistance as shown by total yield of infectious virus (PFU) and cytopathic effect (CPE). In contrast with permissive VERO cells which showed an increase in input PFU to >50/cell, extensive CPE (100%) and HSV-1 DNA replication, both resident PMØ and KC were nonpermissive, showing significant reduction of input PFU to <0.01/cell and no CPE. To further define the block in viral replication in nonpermissive MØ and KC in comparison with permissive VERO cells, Northern blot hybridization was done with cloned probes encoding HSV-1 sequences of immediate-early genes (α4, α0, α22); a delayed-early β2 gene (thymidine kinase,TK), and a true late γ2 gene (glycoprotein C,gC). Both PMØ and VERO cells showed peak viral mRNA levels at 4-6 hrs post infection (pi) for the ICP4, ICP0 and ICP22 genes and at 6 hrs pi for the TK β2 gene. PMØ showed no mRNA for the late gene encoding gC, while VERO cells were positive. VERO cells expressed mRNA at levels far greater than PMØ for all the gene classes positive for MØ mRNA expression. Immunofluorescence assays (IFA) using monoclonal antibodies for HSV-1 α(ICP4), β1 (ICP8) and γ2 (gC) polypeptides were also done. IFA for ICP4 showed peak levels at 4-6 hours pi in VERO cells (95%) and PMØ (15-40%), with KC being negative. IFA for ICP8 showed peak protein levels at 6-8 hrs pi in VERO cells (95%) and PMØ (11-40%). IFA for gC showed VERO cells to be positive, and PMØ to be negative. The results suggest that early transcriptional events are initiated by HSV-1 in resident PMØ, and that the block in replication may be between early and late viral gene expression, while the block in KC is prior to immediate-early gene expression. These data suggest that the intrinsic antiviral resistance mechanisms for PMØ and KC may be different. (Supported by ACSIM-462, AI25751 and AI24004)

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TRANSFORMING GROWTH FACTOR β1 INHIBITS MACROPHAGE ACTIVATION FOR TUMOR CELL KILLING. Mary Haak-Frendscho, Charles J. Czuprynski and Donna M. Paulnock, Depts. Med Microbiol. and Pathobiol. Sci., Univ. Wisc., Madison, WI 53706.

Transforming growth factor β1 (TGFβ1) is a multipotent immunoregulatory peptide that has effects on numerous cell types. Notably, TGFβ1 is able to suppress both T and B cell proliferation as well as NK and LAK cell activity. Here we report that TGFβ1 inhibits the activation of the macrophage cell line RAW 264.7 for tumor cell killing. RAW cells, like freshly isolated peritoneal macrophages, require two signals to become cytotoxic. TGFβ1 inhibits this cytotoxicity in a dose dependent manner at both the priming and the triggering stage. Addition of as little as 1 ng/ml TGFβ1 when added with IFN γ, the priming signal, or when added with LPS, the triggering signal, completely abrogated tumoricidal activity. Target cell lysis increased concominant with decreasing concentrations of TGFβ1. Most or all of the control levels of cytolysis were regained at 0.001 ng/ml TGFβ1. Upon morphological examination, RAw 264.7 cells incubated with TGFβ1 failed to spread and form dendritic processes like those observed in activated control cells. To investigate a possible mechanism for TGFβ1 inhibition of RAW cell cytotoxicity, we examined conditioned media from RAW cell cultures for TNF activity using the L929 cell assay. We found no detectable differences in the amounts of TNF in control versus TGFβ1-treated cell supernatants. In future experiments we will examine IL-1 production and Ia expression. These data suggest that TGFβ1 may be an important mediator in the regulation of macrophage tumorcidal activity.

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TGFβ: DIFFERENTIAL SUPPRESSIVE EFFECTS ON THE ACTIVATION OF MACROPHAGES BY LK AND IFNγ FOR INTRACELLULAR DESTRUCTION OF LEISHMANIA. Barbara J. Nelson, Peter Ralph, and Carol A. Nacy, Walter Reed Army Inst. of Res., Wash., DC 20307-5100 and Cetus Corp., Emeryville, CA 94608.

Transforming growth factor-β (TGFβ) has profound effects on many immunologic reactions involved in host defense. It inhibits T and B lymphocyte proliferation and synthesis of lymphokines and antibodies, and induces differentiation of macrophages during granuloma formation *in vivo*. We examined the effect of TGFβ on the activation of macrophages by LK or IFNγ for intracellular killing of *Leishmania major* amastigotes. Peritoneal macrophages from C3H/HeN mice were infected with para sites and treated with TGFβ and different concentrations of either LK or IFNγ for 72hr. TGFβ at 1 ng/ml blocked (by greater than 50%) the ability of the highest concentration of LK (1/6) to activate macrophages for intracellular killing. This dilution of LK had 30 U/ml IFNγ. In contrast, TGFβ had no suppressive activity on cells treated with recombinant IFNγ. IFN dose responses (30 to 3 U/ml) were identical for cells treated with IFNγ alone or in the presence of 1 ng/ml TGFβ. TGFβ at 100 ng/ml blocked activation of cells by 10 U/ml IFNγ or less, but decreased the killing activity of cells treated with 30 U/ml IFNγ less than 25%. TGFβ at 100 ng/ml totally abrogated the ability of any concentration of LK to induce intracellular killing. TGFβ by itself had no effect on the infection of macrophages by parasites or on the intracellular replication of parasites within macrophages. TGFβ activity blocked macrophage activation at the priming stage. Addition of 10 ng/ml of TGFβ to LK-treated macrophages during the first hour suppressed intracellular killing by 65%; addition of TGFβ at times later than 1 hr had no effect. To determine if TGFβ was responsible for the suppressive effects of EL-4 culture fluids reported previously (J Immunol 133:448, 1984), we treated the fluids with anti-TGFβ antibodies (or an irrelevant antibody as control) and removed the antibody with protein A sepharose. Antibody treatment had no effect on the dose response of EL-4 supernatants for suppression of LK-induced intracellular killing. Thus, we document a potent suppressive effect of TGFβ on macrophages activated by LK, and a 100-fold difference in sensitivity of macrophages activated by IFNγ to this suppressive effect. TGFβ, however,is not responsible for the suppression induced by the EL-4 fluids

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EFFECT OF TRANSFORMING GROWTH FACTOR (TGF) TYPE BETA ON MURINE INFLAMMATORY MONONUCLEAR PHAGOCYTES: INCREASED FIBRONECTIN PRODUCTION Gideon Strassmann, James L. Cone, and Jacqueline Herrfeldt. Otsuka Pharmaceutical Co. Ltd., 9900 Medical Center Drive, Rockville, MD 20850

Transforming growth factor (TGF) type beta has recently been demonstrated to enhance the formation of extracellular matrix proteins in fibroblasts and myoblasts. In this report, the effects of TGF-beta on mononuclear phagocytes (macrophages), cells which play an important role in the inflammatory responses resulting from tissue wounding, was investigated. We found that fibronectin production by murine inflammatory macrophages is significantly enhanced by highly purified human TGF-beta in a time and dose dependent manner. Specifically, 10pM of TGF-beta was sufficient to cause significant elevation of fibronectin levels, which peaked between 24 -48 h of incubation. Both the TGF-beta induced and basal levels of fibronectin were completely abolished by cycloheximide, suggesting that protein synthesis was required. The inductive capacity of TGF-beta appeared specific, since other agents such as phorbol myristate acetate and endotoxin failed to induce fibronectin production. Since macrophages have recently been shown to secrete the inactive form of TGF-beta, the ability of this precursor molecule to induce fibronectin production was tested. It was found that partially purified precursor TGF-beta from human platelets could not induce fibronectin synthesis, whereas TGF-beta released by acid treatment of the same preparation enhanced fibronectin production. Taken together, the results presented here suggest that macrophages can directly contribute to the formation of extracellular matrix upon interaction with TGF-beta, and that these cells lack the ability to respond to the inactive form of TGF-beta in an autocrine fashion.

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TRANSFORMING GROWTH FACTOR BETA INDUCES LEUKOCYTE INFILTRATION AND INFLAMMATION IN THE SYNOVIAL JOINT. Janice B. Allen, Larry Ellingsworth, and Sharon M. Wahl. NIDR, NIH, Bethesda, MD. 20892 and Collagen Corporation, Palo Alto, CA 94303. (Spon: G. Feldman)

Transforming growth factor beta (TGF-β) is a potent bifunctional regulator of the immune response. In vitro, TGF-β induces monocyte and neutrophil chemotaxis at femtomolar concentrations, and picomolar quantities stimulate monocytes to produce inflammatory mediators. Identification of immunoreactive TGF-β in inflamed synovium of rodents with erosive polyarthritis suggested a role for TGF-β in arthritic lesions. In order to define a role for TGF-β in the development of synovitis, TGF-β was injected into the joint space of the hind paws of LEW/N rats. Following the daily intra-articular injection of 1 μg TGF-β1 or its homologue, TGF-β2, marked swelling and erythema of the treated joints were apparent within the first 24 hr. On a scale of 0 to 4, by day 3 the TGF-β treated joints had an articular index (AI) of 3.6±.5 compared to the vehicle injected contralateral joints AI=0 (N=7). Cessation of TGF-β resulted in a gradual decline of the articular index. Histological examination revealed a pronounced infiltration of neutrophils and monocyte-macrophages in the synovium with minimal T cells. The majority of mononuclear phagocytes were Ia^+ indicative of in situ activation. The monocytic pattern of leukocyte infiltration at 2-3 days was comparable to that seen in animals with antigen-induced arthritis after 2-3 weeks. These data suggest that the polypeptide, TGF-β, released by platelets and/or activated inflammatory cells may be a key mediator in leukocyte recruitment and activation in arthritic and other inflammatory lesions.

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GROWTH REGULATION IN LYMPHOPOIESIS AND HEMATOPOIESIS BY TRANSFORMING GROWTH FACTOR-B: REGULATION OF RECEPTOR EXPRESSION: L Ellingsworth, D. Nakayama, and J. Dasch. (Spon: L. Ellingsworth). Collagen Corporation, Celtrix Laboratories, 2500 Faber Place, Palo Alto, CA 94303

The transforming growth factors, TGF-B1 and TGF-B2, are equipotent (picomolar) growth inhibitors of developing and mature lymphocytes and myeloid cells. The anti-proliferative actions of TGF-B1 and TGF-B2 appears to be mediated through a high affinity (Kd = 5-50pm) cell surface receptor complex consisting of three binding proteins (280-200 kD; 95-85 kD and 65 kD). An affinity crosslinking method and neutralizing antibodies to TGF-B1 were used to characterize the expression of these binding proteins on murine thymocytes and splenic T cells. These results show that the TGF-B1 receptor complex is differentially expressed in freshly isolated (resting) and mitogen activated thymocytes and mature T cells. Resting cells were found to constitutively express only the 95-85 kD form of the receptor complex, while mitogenic activation for 12-72 hr was found to induce the appearance of the entire receptor complex (ie., 280-200 kD, 95-85 kD and 65 kD) on both thymocytes and mature T cells. The T cell mitogens interleukin-1, interleukin-2, concanavalin-A and 12-tetradecanoyl-phorbol-13-acetate (TPA) were found to induce the up-regulation of this receptor complex. These observations are consistent with the concept that the TGFBs function to regulate the clonal growth of developing and mature lymphocytes.

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TUMOR NECROSIS FACTOR (TNF) RECEPTORS ON MACROPHAGES (Mϕ) ARE RAPIDLY INTERNALIZED IN RESPONSE TO BACTERIAL LIPOPOLYSACCHARIDE (LPS). A. Ding, E. Sanchez and C. F. Nathan. Cornell Univ. Med. Coll., NY, NY 10021.

Exposure of Mϕ to trace LPS blocks their ability to respond to TNF with an enhanced respiratory burst (J. Imm. 139:1971, '87). We asked if this is due to an LPS effect on TNF receptor (TNF-R). At equilibrium (3-4 h at 4°C), I(125)-rTNFα bound to 1100 ± 200 sites/cell on RAW.264 Mϕ with a kd of 1.3 ± 0.1 x 10E-9 (n = 4). Pre-incubation of RAW mϕ with LPS caused a rapid loss of TNF binding sites on the cell surface without affecting another membrane receptor, complement receptor type 3. This down-regulation was time-, dose-, and temperature-dependent. Incubation with 10 ng/ml LPS for 1 h at 37°C abolished the subsequent binding of I(125)-TNF (IC50, 0.6 ng/ml LPS; t1/2 of inhibition, 15 min), whereas no decrease in TNF-R followed preincubation with LPS at 4°C. Inhibition of I(125)-TNF binding did not appear to be a result of direct occupancy of the TNF-R by LPS since up to 1 μg/ml LPS did not compete with I(125)-TNF at 4°C. Endogenous TNF, induced in response to LPS, did not seem to account for inhibition of I(125)-TNF binding, because little if any TNF is released within 15 min of LPS exposure, high doses (1000 U/ml) of exogenous TNF were required to mimic the LPS effect, and an anti-TNF Ab capable of neutralizing 1000 U/ml TNF did not prevent the LPS effect. Although TNF-R disappeared from the Mϕ surface following exposure to LPS, total TNF-R sites were unchanged in Mϕ permeabilized with digitonin, indicating that TNF-R were rapidly internalized in response to LPS. LPS-induced down-regulation of TNF receptors was also found in mouse peritoneal Mϕ and human endothelial cells, but not in several tumor cell lines. In vivo, Mϕ and certain other host cells may be resistant to the actions of LPS-induced TNF by internalizing their TNF-R before TNF is produced. Thus, the biologic effects of LPS-induced TNF may differ from those seen with TNF alone. (supported by NIH grant #CA43610)

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NOVEL PHAGOCYTIC BEHAVIOR OF HUMAN NEUTROPHILS: SCISSON OF YAC TUMOR CELLS DURING ADCC. M.J. Zhou, J.W. Francis and H.R. Petty. Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

The cellular events accompanying polymorphonuclear leukocyte (PMN)-mediated ADCC directed against YAC target cells have been studied by time lapse fluorescence videomicroscopy. The YAC plasma membrane was labeled with the fluorescent probe diI. YAC cytosol was labeled with the cytoplasmic stain eosin Y (EY). Fluorescently-labeled and IgG-opsonized YAC cells were incubated at 37°C while observed by video intensified microscopy. During temporal studies of PMN-YAC conjugates, YAC cell cytosol was found to accumulate in tubular and spherical compartments of the vacuolar apparatus. To distinguish between several possible mechanisms of target cytosol uptake, diI-labeled YAC cells were observed during identical conditions. The YAC membrane label diI was found to accumulate in PMNs in an identical fashion. At 30 min. 26 to 42% (range) of PMNs in apparent conjugates had internalized tumor cell cytosol or membrane in a vesicular compartment (n = 4 to 6). An artifactual accumulation of EY or diI in PMNs was ruled out by control studies. Non-specific exchanges of EY and diI labels of YAC cells with tannic acid-treated RBCs (TRBC) and normal PMNs was studied. Since hemoglobin binds tightly to EY, RBCs can easily detect EY leakage. No exchange of EY or diI into TRBC of TRBC-YAC rosettes was found. Non-opsonized YAC cell-PMN conjugates were formed using poly-L-lysine. No accumulation of EY or diI in the PMN cytosol or vacuolar apparatus was found, although trace diI labeling of the PMN plasma membrane was noted. Furthermore, direct addition of EY or diI to PMNs labeled cytoplasmic granules poorly or not at all. Our data suggest that PMNs can endocytose tumor cells in a piecemeal fashion by an antibody-dependent mechanism. This micro-scisson phenomenon resembles RBC scission and cell nibbling by lower eukaryotes. It may play a role in target cytolysis and in antigen shedding and/or processing.

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ROLE OF SUPEROXIDE AND ASCORBATE IN THE CYTOTOXICITY OF STIMULATED LEUKOCYTES. Douglas B. Learn and Edwin L. Thomas. Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101.

Reduction of O_2 to the free-radical superoxide (O_2^-) is the first step in stimulated leukocyte O_2 metabolism. O_2^- may play several roles in leukocyte cytotoxicity: 1) O_2^- may act directly as a toxin, reacting with and chemically modifying cellular components, 2) O_2^- undergoes dismutation to yield O_2 and H_2O_2, and H_2O_2 can react directly with target cell components or participate in generation of potent oxidizing and halogenating agents, or 3) O_2^- with H_2O_2 forms toxic metal ion-oxygen complexes. We have proposed a fourth role for O_2^- in leukocyte cytotoxicity, which is the oxidation and removal of reducing agents such as ascorbate from the extracellular environment and the phagolysosome. Ascorbate is the major low-molecular weight reducing agent in plasma and tissue interstitial fluid. Oxidation of ascorbate by O_2^- yields one H_2O_2, double that from dismutation, thus promoting oxidant generation and depleting extracellular reducing capacity which could scavenge and detoxify this increased oxidant burden. Addition of superoxide dismutase (SOD) prevents the reaction of O_2^- with ascorbate, lowering the amount of oxidant generated and preserving the pool of reducing agents. Support for this proposed interaction of O_2^-, ascorbate, and SOD was obtained by incubating stimulated neutrophils with erythrocytes. Low concentrations of ascorbate promoted neutrophil cytotoxicity by increasing neutrophil oxidant generation. However, high concentrations of ascorbate blocked cytotoxicity by reducing the oxidants. Addition of SOD alone had no effect, but SOD and low concentrations of ascorbate provided synergistic protection against neutrophil cytotoxicity. The results indicate that ascorbate modulates the production and cytotoxicity of inflammatory-cell oxidants.

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FUNCTIONAL CHARACTERIZATION OF p120 A MACROPHAGE PROTEIN WHICH COINCIDES WITH TUMORICIDAL ACTIVATION. P. Johnston. The S.R. Noble Foundation, Ardmore, OK 73402.

Macrophage activation for tumoricidal function is associated with changes in gene expression as evidenced by the substantial remodeling of cell protein composi-

tion, including modulation of secretory protein products, cell surface receptors, cell surface antigens defined by monoclonal antibodies, expression of polypeptides analyzed by SDS-PAGE and alterations in the levels of RNA species including ribosomal RNA's as well as specific mRNA species. p120 is a macrophage protein whose expression correlates with the expression of tumoricidal function. An immune serum has been raised which is capable of immunoprecipitating a 120 kDA polypeptide from detergent lysates of p120 expressing cells. The addition of this immune serum does not inhibit the macrophage mediated cytolysis of P815 mastocytoma cells. p120 is not ^{125}I-iodinated under surface labeling conditions when putative macrophage tumor cell binding proteins are labeled, nor is it ^{32}P-phosphorylated in response to activating signals and/or agents capable of modulating the level of protein phosphorylation in macrophages. Under pulse-chase radiolabeling conditions p120 exhibits a relatively long half-life which suggests that the rate of p120 turnover due to either secretion and/or degradation is low.

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TYROSINE KINASE ACTIVATION CONFERS TARGET CELL RESISTANCE TO TNF. T.C. Suen, R.U. Rodriguez, M.-C. Hung, and J. Klostergaard, University of Texas M. D. Anderson Hospital Cancer Center, Houston, TX 77030.

Previous studies have implicated expression of the E1A oncogene in conferring target cell susceptibility to direct cytotoxicity by macrophages (MØ) and NK cells, as well as to lysis by TNF. Since both MØ and NK cells are thought to play a surveillance role at several steps in the metastastic process, we have examined possible mechanisms of subversion of this surveillance related to transformation. Using the NIH 3T3 expression system, we determined that both the parental and H-ras (E.J.) transfectant were susceptible to rHuTNF (Biogen) in the 10^3 - 10^5 units/ml range. Thus, immortalization is irrelevant to the expression of a particular phenotype of TNF-susceptibility; furthermore, an activated p21 is also excluded from a role in this phenotype. However, the transfectant obtained by expression of the v-abl oncogene demonstrated profound resistance to TNF, or even slight growth enhancement, at a dose as high as 7.5 x 10^4 units/ml for as long as 96 hr. This pattern was essentially mirrored in the response to cytotoxic activated macrophages. Thus, activation of tyrosine kinase in target cells during transformation may be one pathway for escape from the surveillance of monocytes/macrophages exerting TNF-dependent cytotoxicity. Supported by NCI CA45265 and ACS CD-360 (M-CH) and ACS IM-419 (JK).

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N^G-MONOMETHYL-L-ARGININE (NMMA) BLOCKS KUPFFER CELL SUPPRESSION OF HEPATOCYTE PROTEIN SYNTHESIS BUT NOT TNF OR IL 1 RELEASE IN RESPONSE TO LPS. T. Billiar, R. Curran, R. Hoffman, B. Bentz, R. Simmons, Univ. of Pittsburgh, Pittsburgh, PA 15261

Endotoxin (LPS) activated Kupffer cells (KC) suppress hepatocyte (HC) protein synthesis (^{3}H-leucine incorporation) in KC:HC coculture through a poorly understood mechanism. The arginine (arg) analog, NMMA, is a specific inhibitor of the arg-dependent mechanism -- a mechanism utilized by activated macrophages to kill tumor cells which occurs through the release of arg metabolites. The table shows that NMMA 0.5mM added with LPS to KC:HC coculture prevented most of the KC induced decrease in HC protein synthesis (PS expressed as cpm±SEM). However, NMMA added with LPS to KC cultured alone did not inhibit but did augment both TNF (units TNF/ml±SEM) and IL 1 (units IL 1/ml±SEM) release as measured 24 hours after exposure to LPS.

		LPS Concentration (ng/ml)			
	NMMA	0	0.1	1.0	10.0
PS	(-)	40714±5425	26159±2845	18486±1301	14866±1062
PS	(+)	43201±1646	35704±881	31614±1068	39163±1996
IL 1	(-)	0	0.028±0.004	0.057±0.002	0.240±0.010
IL 1	(+)	0	0.052±0.007	0.122±0.006	0.432±0.016
TNF	(-)	0	172.8±12.8	216.0±24.1	239.2±20.0
TNF	(+)	0	249.6±73.8	302.4±14.4	304.0±54.6

These data show that the arg-dependent mechanism plays a role in the KC suppression of HC protein synthesis, that IL 1 and TNF have limited roles in the suppression, and that IL 1 and TNF release by LPS-triggered KC is not dependent on the arg-dependent mechanism. The data also suggest that KC may be able to induce HC dysfunction following a septic stimulus through an arg-dependent mechanism.

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CULTURE FLUIDS FROM HIV-INFECTED HUMAN MONOCYTES ARE NEUROTOXIC AND INHIBIT PROLIFERATION OF MITOGEN-STIMULATED LYMPHOCYTES. R. M. Crawford, H. E. Gendelman and M. S. Meltzer. Walter Reed Army Inst. Res., Washington, DC 20307-5100.

Infection with the human immunodeficiency virus (HIV) is associated with profound depletion of CD4+ helper T cells and central nervous system (CNS) disorders. Cells of the monocyte/macrophage lineage are reservoirs of HIV *in vivo* and *in vitro*. Secretory products of such infected macrophages may directly participate in the pathogenesis of immune deficiency and CNS disease. Peripheral blood mononuclear cells (PBMC) from normal human donors were enriched for monocytes and cultured in recombinant human M-CSF (1000 u/ml) and GM-CSF (50 u/ml) for 7 to 10 days before infection with HIV. At various times, fluids from infected and uninfected control cultures were collected and tested in lymphocyte proliferation and neuronal growth assays. Medium alone and control fluids from uninfected monocyte/macrophages had no effect in either assay at concentrations up to 50%. In contrast, fluids from HIV-infected cultures at 0.1% completely abrogated ^{3}H-thymidine incorporation into ConA-stimulated PBMC, and prevented growth and differentiation of cultured neurons. Neuronal toxic activity was relatively selective in its target cell effect: neurons are exquisitely sensitive to the toxic effect, while fibroblasts remain unaffected and brain-derived astrocytes paradoxically proliferate. Participation of HIV-infected macrophages in the immunopathogenesis of CNS disease and CD4+ lymphocyte depletion may be mediated by these secretory products.

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MOLECULAR MECHANISMS OF ANTIGEN INDEPENDENT DENDRITIC CELL-T CELL CLUSTERING. P D King and D R Katz. Univ Coll and Middlesex Sch Med, London W1P 8AA, England.

A feature of dendritic cells is their ability to bind T cells in the absence of exogenous antigen. This antigen independent clustering of T cells by dendritic cells is one of the initial events of dendritic cell-T cell collaboration and is thought to play an important role in the induction of T cell responses. As a model of antigen independent clustering in vitro we have been looking at the binding of human tonsillar T cells to autologous human tonsillar dendritic cells. A sensitive assay of this adhesion has been developed and this has allowed us to examine the molecular mechanisms involved. In this assay, clusters of ^{51}chromium labelled T cells and non-

labelled dendritic cells are encouraged to form in the presence or absence of monoclonal antibodies, over a two hour period at 37°C. Clusters can be separated from free cells by size and the extent of dendritic cell-T cell binding determined by measuring the radioactivity associated with both clusters and free cells. We report here that antibodies specific to CD2, CD18, class II MHC, LFA-3 and ICAM-1 inhibit dendritic cell-T cell adhesion in this system whereas antibodies specific for CD11a, CD25, CD45 and class I MHC do not. The former determinants are thus implicated in antigen independent clustering by dendritic cells and may therefore play an important, early role in the generation of tissue immunological microenvironments.

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LFA-1 AND ICAM-1 IN NEUTROPHIL ADHERENCE AND TRANSENDOTHELIAL MIGRATION. C.W. Smith, S.D. Marlin, R. Rothlein, C.J. Toman, H.K. Hawkins, D.C. Anderson. Baylor Col. of Med., Houston, TX 77054 and Boehringer Ingelheim Pharma. Corp., Ridgefield, CT 06877.

The adherence of human neutrophils (PMNL) to human umbilical vein endothelial cells (HUVEC) is partially dependent on the CD18 family of glycoproteins on the PMNL surface and ICAM-1 on the HUVEC surface. The principal heterodimer in the CD18 family involved in this adherence is unclear. ICAM-1 was purified from SK-hep cells by affinity chromatography using a newly developed anti-ICAM-1 monoclonal antibody (MAb), R6-5-D6. Artificial lipid vesicles containing ICAM-1 or the control protein glycophorin were used to prepare planar membranes on glass coverslips. In a visual adherence assay, isolated PMNL attached readily to the planar membranes containing ICAM-1 (68.6 ± 7.7%, n=10), but not to those containing glycophorin (4.5 ± 2.2%, n=10). Adherence to ICAM-1 was reduced to 17.0 ± 4.4% ($p<0.01$) by pretreatment of the planar membrane with R6-5-D6 (10 ug/ml, $F(ab')_2$), but W6/32 (40 ug/ml, $F(ab')_2$), a MAb of the same isotype recognizing HLA class I antigen, did not reduce adherence (69.5 ± 3.2%). Adherence to ICAM-1 was reduced to 15.6 ± 2.7% ($p<0.01$) and 17.8 ± 3.1% ($p<0.01$) by pretreatment of PMNL with 10 ug/ml of the anti-CD11a MAbs, R3-1 and TS1/22, respectively. In contrast, adherence was not inhibited by pretreatment of PMNL with the anti-CD11b MAbs, M1/70, OKM1, 904 or LM2/1. Similar results for the adherence of PMNL to HUVEC monolayers were obtained using MAbs. Anti-ICAM-1 pretreatment of HUVEC monolayers stimulated with IL-1, and anti-CD11a pretreatment of PMNL each inhibited adherence by >50% ($p<0.01$). When combined in the same experiment, inhibition was no greater than with either alone. In addition, the transendothelial migration induced by 4 hr IL-1 stimulation of HUVEC grown on gelatin-coated glass or amnion was inhibited by >85% ($p<0.01$) by anti-ICAM-1 and anti-CD11a MAbs. These results support the conclusion that LFA-1 and ICAM-1 are important determinants of PMNL attachment and transendothelial migration *in vitro*.

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IMMOBILIZED MONOCLONAL ANTIBODIES SPECIFIC FOR Mol (CD11b/CD18) CAN TRIGGER THE OXIDATIVE BURST OF HUMAN NEUTROPHILS. B.J. Locey, M.D. Adams, C.E. Rogers, and R.F. Todd III. Univ. Michigan Med. Sch., Ann Arbor, MI 48109.

Mol (CD11b/CD18) is an adhesion-promoting leukocyte glycoprotein that serves as a receptor for C3bi (CR3) and for certain other particulate stimuli (e.g., opsonized and unopsonized zymosan) of neutrophil (PMN) secretion and oxidative metabolism. While C3bi-opsonized particles do not stimulate an oxidative burst, zymosan binding to PMNs can trigger a burst response (BR) that is inhibitable by anti-Mol antibodies. To further investigate the role of Mol as a mediator of PMN oxidative metabolism, we determined if immobilized anti-Mol could mimic the stimulatory effect of particulate activators of the BR. For these experiments, anti-Mol monoclonal antibodies (MoAbs) were immobilized on the surface of S. aureus (SA) cell walls either directly (in the case of IgG2a MoAb) or indirectly after pre-opsonization with goat anti-mouse Ig. These opsonized SA particles were found to stimulate the BR of human PMNs (primed to express maximal surface Mol density by preincubation with calcium ionophore A23187) as measured cytofluorographically by the detection of 2',7'-dichlorofluorescein. The BR stimulated by SA-immobilized anti-Mol was rapid (detectable within 2 min and maximal by 15 min) and directly related to the concentration of the stimulus (both the concentration of maximally-opsonized SA and the relative quantity of MoAb per particle). Neither unopsonized SA nor SA opsonized with isotype-identical MoAbs unreactive with the PMN stimulated a BR. To further assess the specificity of the BR to SA-immobilized anti-Mol, the stimulatory effect of other anti-PMN MoAbs (from the Third International Leukocyte Workshop) was examined. Only MoAb specific for epitopes expressed on the α and β subunits of Mol (CD11b, CD18 and CD15) stimulated a significant BR; MoAbs specific for CD11a (LFA-1α), CD11c (p150,95α), CD13 (gp150), CD16 (FcR), CD30 (gp130-140), and CD35 (CR1) were nonstimulatory or triggered a BR of low magnitude. We conclude that SA-immobilized MoAb specific for Mol can mimic the stimulatory effect of particulate activators of PMN oxidative metabolism indicating a role for Mol in transducing BR signals.

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Monocyte Adherence Induces Differential Gene Expression in Monocytes, Endothelial Cells and Stromal Cells. C. Johnson, D. Eierman, S. Haskill, C. Rinehart and C.-J. Edgell, Depts. of Micro. and Immunol., Ob/Gyn., Pathol. and Lineberger CRC, Univ. of N. Carolina, Chapel Hill, NC 27599.

Directed chemotaxis results in monocyte migration into extravascular tissue sites, a process which involves monocyte adherence to endothelial cells as well as extravascular connective tissue cells. We show here the differential effects of interactions between monocytes and endothelial or stromal cells on the regulation of gene expression in both interacting cell types. Monocyte adherence to stromal cells induces high levels of CSF-1, TNFα and IL-1β expression similar to those seen during adherence to plastic. In contrast, levels of CSF-1, TNFα and IL-1β expression induced by adherence to endothelial cells are much lower than those induced by adherence to stromal cells. Expression of lysozyme and c-fms, which are normally down-regulated by adherence to plastic, are similarly regulated by adherence to cellular substrates. In contrast, down-regulation is not regulated by adherence to extracellular matrices. Fibronectin expression, not seen in monocytes at 4h adherence, is elevated above control values in stromal and endothelial cells following monocyte adherence. **We conclude that monocyte adherence to endothelial and stromal cells rapidly affects subsequent gene expression in both cell types. This has important implications for local inflammatory processes.**

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SURFACE CONTACT MODULATION OF INFLAMMATORY MACROPHAGE ARACHIDONIC ACID METABOLISM. P.W. Gudewicz, M.B. Weaver, D.G. Moon and P.J. Del Vecchio. Dept. of Physiology, Albany Medical College, Albany, NY 12208.

We investigated whether inflammatory macrophages adherent to tissue culture plastic, endothelial cell-derived extracellular matrix or gelatin/fibronectin coated surfaces would alter their secretion pattern of arachidonic acid metabolites following phagocytosis. The release of arachidonic acid metabolites from monolayer cultures of rat exudate peritoneal macrophages (PM) that were adherent to the various surfaces for 18 hrs was examined using reverse-phase HPLC. Conditioned media from PM monolayers was collected 1 hr following the addition of serum opsonized zymosan (1 mg/ml) and cyclooxygenase products were separated isocratically and

detected at 203 nm. PM adherent to tissue culture plastic release two major prostanoid peaks following phagocytosis with the first peak eluting with a retention time corresponding to 6-keto-$PGF_{1\alpha}$. The second peak *remains unidentified but appears to be another prostacyclin metabolite.* PM adherent to the extracellular matrix demonstrated a similar HPLC profile with less release of the prostacyclin metabolites. The chromatogram from PM adherent to a gelatin /fibronectin surface showed *a dramatic increase in the release of prostacyclin* metabolites in addition to peaks corresponding to $PGF_{2\alpha}$ and PGE_2. These data support our hypothesis that PM adherent to protein surfaces likely to be found at sites of tissue injury alter their secretion pattern of prostanoids which may *serve as an important feedback mechanism* regulating macrophage function. (Supported by American Heart Grant #87-051G)

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[illegible] OF TUMOR NECROSIS FACTOR ON NEUTROPHIL AND MONOCYTE MIGRATION. I. Schnell-Frederick, I. [illegible], M. [illegible], M. Pfreundschuh, M. Schaadt and V. Diehl. Medizinische Universitätsklinik I, D-5000 Cologne 41, FRG.

In addition to its cytotoxic effects, tumor necrosis factor (TNFα) has multiple effects in normal cells. Among these TNFα has been reported to stimulate superoxide production directly in neutrophils but not in monocytes and to prime both cell types for increased oxygen metabolism. TNFα has been reported to be chemotactic for neutrophils and monocytes. In our hands rHTNFα (Asahi Chemical Industry, 2.2×10⁶ U/mg), in vitro, was not chemotactic for either human neutrophils or monocytes, although it produced the expected effects on neutrophil superoxide production. Neutrophils preincubated with rHTNFα in concentrations above 1 U/ml demonstrated inhibited random migration and migration toward standard chemoattractants (FMLP, Zymosan-activated plasma). Normal monocytes preincubated for up to [illegible] hours showed normal migration. Initial results in Rebuck skin window also showed no direct chemoattractant effect of rHTNFα (100-10,000 U/ml). Evaluation of other rHTNFα preparations and of rHTNFβ are in progress in order to rule out the possibility that our results are limited to one rHTNF. In further studies we have measured migration and superoxide production in neutrophils and monocytes isolated from patients receiving rHTNFα in a phase II clinical trial (30 minute i.v. infusion of [illegible]×10⁵ U/m²). Neutrophil migration was maximally inhibited at 30 minutes coinciding with the peak TNF blood level. At this time basal whole blood superoxide production (corrected for absolute neutrophil and monocyte count) was markedly [illegible] and no further [illegible] was seen in the [illegible] of phorbol myristate acetate. Patient monocytes, [illegible] treated inhibited migration, but only at later [illegible] ([illegible] hours). Rebuck skin windows in patients are currently being performed. The question whether TNF [illegible] chemotactic [illegible] clearly an important one for [illegible] mechanisms of cytokine action and of tumor [illegible], and for [illegible] the therapeutic [illegible] of rHTNF. Our data suggest that when TNFα is [illegible] in the circulation, neutrophils and monocytes [illegible], partly at [illegible] [illegible] [illegible] to migrate to [illegible] sites.

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CHARACTERIZATION OF THE DEFECTIVE P/J MOUSE MACROPHAGE RESPONSE TO ACTIVATION SIGNALS. Anne H. Fortier, David S. Finbloom and Carol A. Nacy. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC. 20307-5100.

Macrophages from P/J mice are unresponsive to lymphokine (LK) signals for aquisition of potent killing effector functions: they fail to respond to LK for either extracellular tumor cytotoxicity or intracellular parasite destruction. The best characterized macrophage activating factor in LK preparations is gamma interferon (IFN). And, P/J mouse macrophages are equally unresponsive to purified or murine recombinant IFN for induction of effector functions of activated macrophages: only 10% microbicidal activity (measured as a reduction in Leishmania infected macrophages 72 hr after treatment with stimulus) observed in cultures of P/J mouse macrophages treated with 60 U/ml IFN compared to 90% microbicidal activity observed in LK responsive macrophages from C3H/HeN mice. This profound macrophage defect was not the result of defective killing mechanisms, since macrophages from Leishmania infected P/J mice, when challenged in vitro with intracellular parasites, were 80% microbicidal after 72 hr. We used IFN to induce another functional *response in macrophages, one that is unrelated to killing activity,* to confirm that the defect was not at the level of effector mechanisms. IFN treatment of bone marrow macrophages induces increased Fc receptor (FcR) expression. Bone marrow macrophages from LK-unresponsive P/J mice responded to 100 U/ml IFN with a 1.5-2 fold *increase in FcR binding compared to a 3-5 fold increase observed in* similarly treated bone marrow macrophages from LK-responsive C3H/HeN mice. It would appear that the defect in P/J mice is quantitative rather than qualitative since the macrophages have the effector mechanisms but respond marginally or only during on *ongoing immune response. To determine if decreased responsiveness to* IFN for induction of effector functions was at the receptor level, we performed absorption studies with P/J and C3H/HeN mouse macrophages. Macrophages from P/J mice incubated with IFN or IFN containing hybridoma supernatants at 4°C absorbed 60-100% of the IFN activity. Responsive C3H/HeN macrophages similarly absorbed IFN activity from these supernatants. Studies in progress are aimed at determining whether the defect is at the level of signal transduction after receptor-ligand interaction.

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TRANSGLUTAMINASE LEVELS AND IMMUNOLOGIC FUNCTIONS OF BCG-ELICITED MOUSE PERITONEAL MACROPHAGES ISOLATED BY CENTRIFUGAL ELUTRIATION. V. Khera[1] and K. Mehta[2]. University of Texas Medical Branch[1], Galveston, TX 77550 and UT MD Anderson Hospital[2], Houston, TX 77030.

BCG-elicited mouse peritoneal macrophages were separated into three subpopulations by counterflow centrifugal elutriation. The three subpopulations were characterized on the basis of a protein cross-linking enzyme level, tissue transglutaminase and the enzyme activity was measured as calcium dependent incorporation of [^{3}H]-putrescine into dimethyl casein. Subpopulation three consisted of large cells (>95% esterase positive and >90% viable) and had at least fivefold higher transglutaminase activity (35 ± 6 nmol/hr/mg) as compared to macrophages in subpopulation one (6 ± 2 nmol/hr/mg) and at least threefold higher activity as compared to subpopulation two (11 ± 2 nmol/hr/mg). Subpopulation three also showed sevenfold higher phagocytosis of IgG coated sheep red blood cells. The three subpopulations showed no difference in their ability to kill Listeria monocytogenes as determined by [^{3}H]-thymidine release. Subpopulations two and three caused 90% inhibition of murine adenocarcinoma (EMT-6) tumor cell growth in presence and/or absence of detoxified Salmonella lipopolysaccharide (LPS). Subpopulation one had poor ability to inhibit EMT-6 cell growth (29 ± 12%). However, in the presence of detoxified Salmonella LPS this activity increased by at least threefold (92 ± 7%). The three subpopulations exhibited no significant difference in their cytolytic activity against murine mastocytoma (P815) target cells, in the presence and/or absence of LPS. These results suggest that tissue transglutaminase may have no significant role in bactericidal, tumoricidal or tumorstatic function of macrophages, but might have some role in promoting the Fc-receptor mediated phagocytic function of the macrophages.

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DIFFERENTIAL EFFECT OF RECOMBINANT GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) ON HUMAN MONOCYTES AND ALVEOLAR MACROPHAGES. M.J. Thomassen, B.P. Barna, H. Wiedemann, M. Farmer, R. Bukowski and M. Ahmad. Cleveland Clinic, Cleveland, OH 44106

GM-CSF has been demonstrated to be a pluripotent cytokine capable of modulating activities of both immature and mature granulocytes and monocytes. Although the effect on blood monocyte tumoricidal activity has been evaluated, the possible influence of GM-CSF on human

alveolar macrophage activity has not been examined. We compared the tumoricidal activity of alveolar macrophages and monocytes from normal volunteers before and after exposure to GM-CSF or interferon-gamma. Cytotoxicity was assessed using ^{3}H-thymidine labelled human tumor cells (SKMEL 28, melanoma). Neither alveolar macrophages nor monocytes exhibited significant cytotoxicity when incubated in media alone. Recombinant interferon-gamma (Biogen) induced equivalent tumoricidal activity in monocytes and alveolar macrophages (55.6 ± 13.8, mean % cytotoxicity ± SE; 54.9 ± 9.9, respectively). However, a dose-response study (50-5000 U/ml) of recombinant GM-CSF (Genetics Institute) indicated dramatic differences between cytotoxicity of alveolar macrophages and blood monocytes. Responses of GM-CSF treated alveolar macrophages were different at 1000-5000 U/ml from media controls ($p < .001$), whereas monocytes were not different from controls at any doses tested. Differences between alveolar macrophage and monocyte responses were most pronounced at 5000U/ml GM-CSF where alveolar macrophage cytotoxicity = 41.6 ± 6.5, n = 7 and monocyte cytotoxicity = 8.8 ± 3.1, n = 5 ($p = .004$). The differential effect of GM-CSF on alveolar macrophage and monocyte tumoricidal activity suggests intrinsic differences in ability to respond to this cytokine.

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DIFFERENTIAL EFFECTS OF LIPOSOME-INCORPORATION ON LIVER MACROPHAGE-ACTIVATING POTENCIES OF LPS, LIPID A AND MDP; DIFFERENCES IN SUSCEPTIBILITY TO LYSOSOMAL ENZYMES.

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[1]University Groningen, The Netherlands and [2]Veterans Administration Medical Center, West Haven CT 06516, U.S.A.

We investigated the in vitro activation of rat liver macrophages (Kupffer cells) to a tumor-cytotoxic state with muramyl dipeptide (MDP), rough lipopolysaccharide (LPS) and lipid A both in free and liposome-encapsulated form. The tumor cytotoxic state of the liver macrophages was determined with a [methyl-^{3}H]thymidine release assay using C26 colon adenocarcinoma cells as target cells.

As was shown previously,(T. Daemen et al., Cancer Res.46 (1986) 4330-4335) the encapsulation of MDP within multilamellar phospholipid vesicles greatly enhanced the activating potency of the drug towards liver macrophages; by contrast, encapsulation of LPS or lipid A according to Dijkstra et al. (J. Immunol. 138 (1987) 2663-2670) significantly reduced the activation of macrophages as compared to the free form of these agents. At a dose of 1 ng of free LPS per ml a significant induction of tumor cell lysis was observed while a maximal level was obtained at a concentration of approximately 10 ng per ml. By encapsulation of LPS in liposomes the activating potency diminished 20 to 100-fold. The minimal concentration required to induce detectable macrophage activation with free lipid A was 10 ng per ml, while liposome-encapsulated lipid A did not induce any detectable tumor cell lysis upto a concentration of 200 ng per ml. After a 1 h preincubation with a lysosomal fraction from rat liver at pH 4.8, the macrophage-activating potency of LPS and lipid A was diminished by upto 95% whereas MDP remained fully active under these conditions. We conclude that, due to endocytic uptake of liposome-incorporated LPS and lipid A and subsequent intralysosomal degradation, these immunomodulators are inactivated with respect to their potency to activate liver macrophages to tumorcytotoxicity.

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INTERLEUKIN 2 AND INTERFERON GAMMA ACT SYNERGISTICALLY TO PRIME AND ACTIVATE KUPFFER CELLS. R. Curran, T. Billiar, B. Bentz, F. Ferrari, R. Simmons. Univ. of Pittsburgh, Pittsburgh, PA 15261.

Lymphokines, specifically interferon-gamma (IFN-g), prime and activate macrophages (MØ). Although MØ are known to express interleukin 2 (IL 2) receptors, the effect on IL 2 of MØ function is unknown. We have previously presented evidence suggesting that IL 2 is capable of priming Kupffer cells (KC). In vitro KC, the specialized MØ of the liver, significantly suppress hepatocyte (HC) protein synthesis when activated by endotoxin (LPS). In this investigation we studied the ability of IFN-g and IL 2 to cooperate in priming and activating KC. We found that 1.0 U/ml of IFN-g and IL 2, which independently had no effect on KC activation, together significantly primed KC, reducing the concentration of LPS required for KC activation by 1.5 $\log_{10}$ dilutions (see table). More importantly, we found that higher concentrations of IFN-g and IL 2, which alone had no affect, together activated KC in the absence of LPS. KC exposed to 100 U/ml of IFN-g and 500 U/ml of IL 2 were activated to suppress HC protein synthesis to 25% that of control HC ($p \leq 0.001$). These data show that IFN-g and IL 2 act synergistically to prime KC, and at high concentrations even activate KC. These data also suggest that IFN-g and IL 2 cooperate in regulating or modulating KC/MØ responses to septic stimuli.

LYMPHOKINE EFFECTS ON KC MEDIATED ALTERATIONS IN HC PROTEIN SYNTHESIS
(expressed as % control HC Protein Synthesis ± SEM)

Lymphokine (U/ml)	LPS Concentration (ng/ml) 0	1.0	5.0	10	100
0	154±7.3	149±4.9	150±3.2	132±4.8	60±3.5
IFN-g 1.0	153±6.8	146±4.3	146±6.0	140±4.2	50±4.2
IL 2 1.0	144±6.0	152±6.8	151±8.0	140±9.7	81±6.7
IFN-g 1.0+ 1.0 IL 2	149±5.9	134±3.9	*64±6.0	*33±3.1	*22±3.4

* $p < 0.01$ suppression compared to all others.

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DECREASED EXPRESSION OF IMMUNE ASSOCIATED (Ia) *ANTIGEN AND PRODUCTION OF TUMOR NECROSIS FACTOR* BY LIPOPOLYSACCHARIDE ACTIVATED LIVER MACROPHAGES. T.W. McCloskey, C.R. Gardner, F.M. Robertson and D.L. Laskin. Rutgers University and UMDNJ-Robert Wood Johnson Medical School, Piscataway NJ 08854.

Treatment of rats with lipopolysaccharide (LPS) results in the accumulation of activated macrophages in the liver. These cells display altered morphology, and enhanced phagocytosis, chemotaxis, cytotoxicity and release of reactive oxygen mediators. In the present studies we determined if LPS macrophages are also immunologically activated, i.e. have the potential of modulating lymphocyte activity. Macrophages were isolated from livers 24 hr after treatment of rats with 5 mg/kg LPS or control by combined collagenase/pronase perfusion followed by differential centrifugation on a metrizamide gradient. Ia antigen expression was quantified by flow cytometry and indirect immunofluorescence using a highly specific monoclonal antibody. Release of tumor necrosis factor (TNF) into the culture medium from liver macrophages was measured by cytotoxicity towards actinomycin D sensitized L929 cells. We found that both resident Kupffer cells (RKC) and LPS activated liver macrophages (LKC) expressed Ia antigen. Antibody binding was uniformly distributed on the surface of the cells. However, using flow cytometry, we found that expression of Ia antigen on LKC was two fold less than on RKC. In addition RKC were found to produce TNF in a time dependent manner reaching a maximum 4-6 hr after macrophage isolation. In contrast, LKC did not produce significant quantities of this mediator at any time in culture. These results suggest that factors in addition to LPS are required for immunological activation of liver macrophages. Supported by NIH grant GM34310.

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cAMP SELECTIVELY SUPPRESSES LPS-INDUCED MACROPHAGE GENE EXPRESSION. C.S. Tannenbaum and T.A. Hamilton, Cleveland Clinic Foundation, Cleveland, OH 44195.

Elevation of intracellular cAMP has been associated with the suppression of macrophage activation. The present study has examined the effects of agents which alter levels of cAMP on LPS-induced macrophage gene expression. Treatment of murine peritoneal macrophages with LPS leads to dramatically enhanced expression of multiple gene products including the competence genes JE and KC and the inflammatory monokines IL-1 and TNF. If macrophages are first treated with cholera toxin or dibutyryl cAMP 15 min prior to stimulation with LPS, the accumulation of mRNA encoding both JE and TNF is strongly suppressed while mRNA levels for KC and IL-1 are unaffected. The suppression of JE and TNF mRNA levels is dose dependent, being maximal at 100 μM dibutyryl cAMP; concentrations as high as 1 mM do not affect the expression of either KC or IL-1. When dibutyryl cAMP was added after initiation of LPS treatment, suppressive effects diminished in a time dependent fashion. Furthermore, cAMP blocked LPS-induced transcription of the TNF gene. LPS-induced expression of JE is mediated by hydrolysis of polyphosphoinositides and involves a post-transcriptional mechanism. Treatment with dibutyryl cAMP suppressed JE expression induced by treatment with phorbol ester and A23187 indicating that the inhibitory mechanism operates after the initial transmembrane signalling event. Finally, dibutyryl cAMP only marginally affected the constitutive transcription of the JE gene indicating that suppression acts at a post-transcriptional level. These results indicate that the suppressive effects of elevated cAMP in macrophages are mediated in part through inhibition of early gene expression and that such effects may be pleiotropic involving multiple molecular mechanisms. Supported in part by USPHS grant CA39621.

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ACTIVATION OF MONONUCLEAR PHAGOCYTES BY BACTERIAL PEPTIDOGLYCANS: POSSIBLE ROLE IN THE PATHOGENESIS OF ARTHRITIS. M. Poy, J. Allen, H. Wong, L. Wahl and S. Wahl. NIDR, NIH, Bethesda, MD 20892. (Spon: G. Feldman)

Inflammatory arthropathies are characterized by leukocyte infiltration and synovial hyperplasia. Activation and altered functions of mononuclear cells in the synovium are likely associated with disease progression and abnormal immunoregulation. Bacterial peptidoglycans (PG) are known potent polyclonal activators and have been identified in the synovium of septic arthritis patients and some other arthropathies. Since PG have been localized within synovial macrophages, we investigated the effect of group A streptococcal cell walls containing PG on monocyte phenotype and function. Human peripheral blood monocytes, purified by countercurrent elutriation, were exposed to PG in vitro and analyzed for expression of activation markers including HLA-DR and IL2 receptors by flow microfluorometry. The expression of IL-2R on >50% PG treated monocytes within 24-48hr correlated with cell maturation as indicated by an increase in HLA-DR expression. PG-stimulated monocytes also produced significantly increased levels of inflammatory mediators including prostaglandin E_2, tumor necrosis factor, interleukin 1 and fibroblast activating factor, which have been suggested to be critical mediators in various chronic arthropathies because of their ability to induce synovial cell proliferation and activation. These data suggest that bacterial PG may directly activate mononuclear phagocytes and induce them to secrete inflammatory mediators which may be instrumental in the evolution of synovial cell proliferation, activation and joint destruction characteristic of arthritic lesions.

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THE CONDENSED TANNIN FROM COTTON MILL DUST PROMOTES THE RELEASE OF ARACHIDONIC ACID FROM RABBIT ALVEOLAR MACROPHAGES. M. Rohrbach, T. Kreofsky, J. Russell (Spon: R. Ritts). Mayo Clinic, Rochester, MN 55905 and SUNY-Buffalo, Buffalo, NY 14218

Condensed tannin is a major botanical component of the occupational air pollutant, cotton mill dust. Inhalation of tannin by experimental animals results in an acute pulmonary inflammatory response that includes the release of arachidonic acid (AA) metabolites into the airways. Since alveolar macrophages contain significant amounts of AA in their plasma membranes, the occurrence of AA metabolites in the airways in vivo could be due to tannin-mediated release of AA from these cells. To test this hypothesis, rabbit alveolar macrophages were isolated by bronchoalveolar lavage and labeled in vitro with [^{14}C]-AA for two hours. Approximately 80% of the incorporated [^{14}C]-AA was found in four membrane phospholipids, phosphotidyl choline (PC), phosphotidyl ethanolamine (PE), phosphotidyl inositol (PI) and bis-monoacyl phosphotidic acid (BMAPA). The labeled cells were then challenged with tannin. Challenge with zymosan served as a positive control. Tannin caused a time and dose-dependent release of AA into the culture media. Maximal release ranging from 12 to 28% of the incorporated AA occurred after a 2-hour exposure to 100 μg tannin/mL. This release could be inhibited in a dose-dependent manner by the protein synthesis inhibitor, cycloheximide, similar to that seen with zymosan-stimulated release. Analysis of the membrane phospholipids demonstrated that the tannin-mediated release of AA was accompanied by a statistically significant decrease in the amount of AA-labeled PC (37% decrease, $P<0.020$) and PI (54% decrease, $P<0.025$). These results demonstrate that tannin can evoke the release of AA from selected membrane phospholipids of rabbit alveolar macrophages in vitro. The properties of this release are compatible with tannin-induced secretion of AA metabolites into the airways seen in vivo. (Supported by NHLBI grant HL-28669).

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HUMORAL FACTORS IN THE INDUCTION OF PROSTAGLANDIN E_2-PRODUCING MACROPHAGES IN VITRO. Y. SHIBATA. Dept. of Pathology, East Carolina University School of Medicine, Greenville, NC 27858, and Fujisaki Cell Center, Hayashibara Biochemical Laboratories, Inc., Okayama 702, Japan.

Bone marrow macrophages (Mφ) from CBA/J mice were incubated 24 hours in media enriched with normal mouse serum and then stimulated with calcium ionophore A23187, phorbol ester (PMA) or zymosan (Zy) for 2 more hours. PGE_2 release promoted by each agent was almost 10 times higher than from control Mφ cultured without serum or with sera from other species such as rat, bovine, rabbit and human. Maximum release of PGE_2 was 520ng/mg protein (A23187); No release of leukotriene C_4 (<10ng/mg) was detectable. Mouse serum enhancement of marrow Mφ for PGE_2 release with A23187, PMA and Zy was found to be dose dependent. Cellular phospholipase A_2 activity was significantly enhanced by serum. These enhancing activities of mouse serum were nondialyzable, inactivated by incubation at pH 2 for 24 hours, sensitive to pepsin digestion, stable at 56°C for 30 minutes, and could not be replaced by defined cytokines such as IL-1, IL-2, IL-3, IFNα/β, IFNγ, TNF, CSF-1 or GM-CSF. In contrast with marrow Mφ, thioglycollate-elicited peritoneal Mφ, which normally release only small quantities of PGE_2, showed over 5-fold increases in PGE_2 production following treatment with IFNγ, IFNα/β or TNF, but not with other cytokines or mouse serum. These data show stable differences in eicosanoid

metabolism between Mφ which suggest, in turn, highly independent regulatory mechanisms in this pathway.

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ASSESSMENT OF MACROPHAGE INFILTRATION AND MATURATION IN THE GUINEA PIG LUNG IN RESPONSE TO AN ACUTE INFLAMMATORY STIMULUS. D.K. Newton-Nash and P. Abramoff. Marquette University, Department of Biology, Milwaukee, WI 53233.

Later stages of acute inflammation are characterized by elevated levels of activated alveolar macrophages (mø). To characterize the timing, localization and extent of mø infiltration and activation during acute pulmonary inflammation, guinea pigs were immunized, boosted and aerosol challenged with ovalbumin, then killed at 12, 24 or 48 hours (hr) postchallenge (PC). Normal animals served as controls. Alveolar mø (AM) and lung tissue mø (LTM) were recovered by bronchoalveolar lavage (BAL) and collagenase digestion of lavaged lung tissue (LT), respectively. Mø in recovered cell populations were identified by staining cytocentrifuged cells for alpha-naphthyl-acetate esterase activity. A decrease, relative to normal values in the percent of total cells which were mø was observed over the first 24 hr of inflammation in BAL and LT digests. In contrast, the number of LTM recovered increased at 12 hr PC and then decreased, while the number of AM recovered increased through 48 hr PC. Immature mø were identified by positive cytochemical staining for peroxidase activity (PRX^+). Increases, relative to normal values, in the percent and number of PRX^+ LTM and AM were observed at 12 hr PC. Increases in PRX^+ AM numbers were observed through 48 hr PC, while PRX^+ LTM decreased to normal values at this time. Mature mø were identified by acid phosphatase positivity (ACP^+). The percent and number of ACP^+ LTM did not show increases relative to normal values. In contrast, while the percent of AM which were ACP^+ did not change over the course of inflammation, the number of ACP^+ AM increased, relative to normal values, at all PC times. Maturation of C3b-receptor function was also used to assess mø maturity. The proportion of LTM and AM capable of ingesting complement-coated erythrocytes (EIgMC) dropped, relative to normal values, at 12 hr PC, then rose through 48 hr PC. The number of EIgMC ingested per phagocytically active cell showed a similar pattern. These data suggest that immature mø accumulate in the lung within 12 hr after acute inflammation initiates, then mature over the next 48 hr, with the highest level of maturity reached in AM.

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COMBINATION OF C-REACTIVE PROTEIN (CRP) AND INTERLEUKIN-2 ENHANCES HUMAN MONOCYTE TUMORICIDAL ACTIVITY. B. Barna, M.J. Thomassen, S. Malcolm-Kohn, J. Pettay and S.D. Deodhar. Cleveland Clinic, Cleveland, OH 44195-5131.

We have observed that endotoxin-free native human CRP enhances the tumoricidal activity of normal human monocytes (MOs) (Cancer Res 47:3959, 1987) and alveolar macrophages (AMs) in a dose-dependent manner. Because Interleukin-2 (IL-2) has also been reported to enhance MO tumoricidal activity, we hypothesized that a combination of CRP and IL-2 might result in greater activity than that resulting from either agent alone. Normal MOs or AMs were exposed in vitro for 24 hrs to CRP (25-100 µg/ml), recombinant (r) IL-2 (20-500 U/ml) [Hoffman LaRoche], a combination of rIL-2 and CRP, or medium alone. Percent cytotoxicity (CTX) was determined after 48-96 hr co-culture with ^3H thymidine-labelled SK-MEL-28 melanoma cells at a 10/1 effector/target ratio. CRP enhanced CTX of both MOs and AMs but 500 U/ml rIL-2 alone elevated CTX of MOs (27 ± 3.5% SEM) [$p<0.001$] and not AMs (6.3 ± 2.7%) compared to medium controls (MO = 1.8 ± 0.9%; AM = 4.0 ± 1.8, n = 4 experiments). In MOs, tumoricidal activity was elevated to a greater degree by rIL-2-CPP combinations than by either reagent alone. Similar elevation was not seen with AMs at the doses tested. The combination of 100 U/ml rIL-2 and 100 µg/ml CRP yielded higher MO CTX (44.5 ± 4.6%) than either 100 U/ml rIL-2 (16.8 ± 4.3%) or 100 µg/ml CRP (28.0 ± 7.9) alone (n = 4 experiments). MOs exposed to these reagents did not lyse ^{51}Cr-labelled, natural killer (NK) sensitive K562 cells in a 4 hr assay; further, treatment of MOs with anti-NK sera (anti-Leu 11b) + C' did not abrogate CTX induced by rIL-2-CRP combination, suggesting that NK activity was not involved. These observations which indicate that a combination of CRP and rIL-2 potentiates the antitumor activity of MOs in vitro, may have significant therapeutic implications.

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REGULATION OF MACROPHAGE ACTIVATION BY A COLONY STIMULATING FACTOR G.Frendl and D.I. Beller (Spon.: C.L. Miller)

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It has been previously reported that colony stimulating factors (CSFs) (specifically GM-CSF and CSF-1/M-CSF)have the ability to prime macrophages for tumor killing. Recently Falk et al. have shown that bone marrow monocytes, cultured in GM-CSF , express higher *levels of Ia mRNA and surface Ia antigen.* Their study indicates a distinction between monocytes that develop from bone marrow under the influence of different CSFs.

We were interested in studying the effect of members of the CSF family on the functional status of fully mature murine macrophages (peritoneal *exudate cells*;PEC), cells which only have a limited proliferative capacity to CSFs. We have identified one member of the CSF family as a potent activator of fully mature macrophages, whereas the others had either marginal or no activity in the functional assays that were assessed. This CSF, possessing macrophage activating properties,induced Ia expression on the cell surface to comparable degree with interferon-gamma (IFN-gamma), and showed a delayed *kinetics of induction*,as well as high dose inhibition,not seen with IFN-gamma at comparable doses(unites). This CSF regulates the expression of both the IA and IE subloci. It is also able to induce LFA -1 expression on the surface of PECs with a higher potency than IFN-gamma *itself* , *based on equivalent units of* activity,although the maximal level of LFA-1 expression in response to both stimuli is equivalent. In contrast to the activity of this mediator in both Ia and LFA-1 induction , it is unable to induce macrophage tumoricidal activity. The macrophage activating CSF via its ability to induce the expression of cellular interaction molecules (Ia and LFA-1) , while lacking the ability to induce cytotoxicity , provides a unique form of macrophage activation that serves as an amplification mechanism for cellular interactions.

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BRIEF EXPOSURE TO Ca^{++} CAUSES INCREASED EXPRESSION OF MEMBRANE COMPLEMENT RECEPTORS AND ADHESION MOLECULES BY HUMAN PERIPHERAL BLOOD MONOCYTES AND LYMPHOCYTES ISOLATED *IN THE PRESENCE OF* EDTA.

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Peripheral blood mononuclear cells from EDTA anti-coagulated blood were separated by density gradient centrifugation; then stored in Ca^{++} and Mg^{++} free Dulbecco's PBS at 4°C and cell surface markers measured before and after exposure to $CaCl_2$ (2 mM) for 5 min. at 37°C, by quantitative immunofluorescence analysis on flow cytometer. Complement receptors I (CR1:CD35) and 3 (CR3-CD11b) were detected using monoclonal antibodies (MoAb) 44D and OKM1, respectively; adhesion molecules LFA-1 (CD11a) and p150,95 (CD11c) with MoAb IOT16 and LeuM5, respectively; Fc gamma receptors I, II(CD32), and III(CD16) with MoAb 32.2, IV.3, and 3G8 respectively. Data are expressed as MSF (mean specific fluorescence-linear fluorescence intensity of specif-

ically labelled cells minus the mean non-specific fluorescence). Exposure of monocytes to $CaCl_2$ resulted in dramatic two fold increases in the numbers of CR1 and CR3, and of adhesion molecules LFA-1 and p150,95 expressed per cell. Thus, the MSF of CR1 and CR3 increased from 15 to 30 and from 100 to 200, respectively; and the MSF of LFA-1 and p150,95 both increased from 30 to 60. In contrast, there were no changes in the numbers of Fc gamma receptors I, II, III expressed per monocyte (MSF's stayed at <1,45,15, respectively). Exposure of lymphocytes to $CaCl_2$ resulted in dramatic 2.5 fold increses in the numbers of CR3 and LFA-1 expressed per cell (MSF up from 4 to 10 and 10 to 25, respectively), but no change in the numbers of CR1 and p150,95 (MSF's of 2 and 2) expressed per lymphocyte; and again the numbers of Fc gamma receptors I, II, III remained constant (at MSF's of <1, 2, 45). The rapid time course of the increases in numbers of labelled surface molecules suggests that Ca^{++} is triggering the mobilization of intracelluiar pools of complement receptor and adhesion molecules. The nature of this process and any concommitant fuctional changes are being investigated.

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DOWN REGULATION OF INTERFERON(IFN)-α AND IFN-β ON MACROPHAGE ACTIVATION BY IFN-γ. P.Vitale*, A.Misefari, E.Jirillo, V.Covelli* and S.Antonaci*, Chairs of Immunology, University of Messina Med.School, Messina and Immunology, Clinical Neuroanatomy and Clinical Medicine, University of Bari Med.School, Bari, Italy.

The antiviral activity of Interferon (IFN)-α and IFN-β as well as the activation of macrophage microbicidal and tumoricidal effects of IFN-γ have been extensively reported by several investigators. However evidence is presented here that IFN-α and IFN-β may play a depressing effect on the development of the antimicrobial activity of macrophages induced by IFN-γ. Murine proteose-peptone elicited peritoneal macrophages were precultured for 12 to 24 hrswith plain medium of with IFN-α (up to 200 U/ml). The rate of intrecellular killing of Listeria monocytogenes following macrophage treatment with IFN-γ (0.1 to 100 U/ml) was then evaluated. Macrophages pretreated with IFN-α showed significantly lower levels of bactericidal activity as compared with macrophages cultured in medium alone, suggesting that they were less sensitive to activation by IFN-γ. Pretreatment of macrophages with IFN-β gave very similar results. Addition of neither exogenous Prostaglandin (PG) E2 (up to 10^{-4} M) nor of endogenous PGE2 inhibitor indomethacin (10^{-5} M) influenced the depressing effect of IFN-α and IFN-β, suggesting that PG patway is not involved in such a system. The data reported indicate that IFNs may play, at least in vitro, a divergent role in the regulation of macrophage activation against intracellular microrganisms. Further studies are in progress to better clarify this phenomenon.

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IRON-LOADING INCREASES IL-1 SECRETION IN P388D1 CELLS BY DECREASING MEMBRANE ASSOCIATED ACTIVITY. S. Shedlofsky, C. McClain, D. Cohen, J. Robinson, and K. Keaton. VA Hospital and Univ. Kentucky, Lexington, KY 40511.

Because iron metabolism is linked so closely to macrophage function, and there is evidence that iron-loaded macrophages of patients with iron-storage diseases might contribute to organ dysfunction, we looked at the effects of iron-loading and iron-depletion on IL-1 release from murine P388D1 cells. Confluent cells in RPMI medium with 1% FCS were stimulated with 20µg/ml LPS (E. coli 0111:B4) in the presence of either 100µM nitrilotriacetate (NTA), 100µM ferric-NTA (FeNTA) or 178µM desferrioxamine (DES). After 24h, the medium was harvested, the cells were sonicated and the membranes separated from cytosol by spinning at 12,000g. The pellets were solubilized in SDS-NaOH. All samples were then dialyzed vs. medium in 6-8K MW exclusion tubing and then tested in a standard LAF assay. Data below are from 1:16 dilutions of samples which were brought to equal volumes before testing. N = 3 and SDs are shown.

	CPM X 10^3*		
	Medium	Cell Pellet	Cytosol
NTA Control	15.1 ± 1.4	29.2 ± 3.7	49.3 ± 1.0
FeNTA	22.4 ± 0.7	13.5 ± 1.2	47.9 ± 3.7
DES	14.7 ± 0.5	28.7 ± 0.3	46.2 ± 1.0

Transmission EMs showed ferritin granules and siderosomes in the FeNTA-treated cells that were not found with NTA or DES. Most LAF activity was found in the cell cytosol and treatments did not alter activity. With FeNTA there was more LAF activity secreted into medium and less membrane-associated LAF. DES did not seem to affect LAF activity.

Conclusion: Iron-loading of cultured P388D1 macrophages causes more LPS-stimulated IL-1 to be secreted and decreases the amount of membrane associated IL-1. Iron-depletion with the Fe^{3+}-chelator desferrioxamine does not seem to affect IL-1 release in these cells.

***Neg LAF control =3500 ±200; Pos control =58,177 ±4921 CPM**

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DOWN REGULATION OF HUMAN PERIPHERAL MONOCYTE INTERLEUKIN-1 BY INTERLEUKIN-4. R.Essner, J.S.Economou, K.Rhoades, W.McBride, and D.L. Morton. Div. Surgical Oncology, Factor Bldg., UCLA Medical Center, Los Angeles, CA 90024.

Originally described as a B-cell stimulatory factor, Interleukin-4 (IL-4) can modulate the activity of a variety of cells of the hematopoietic lineage. Human peripheral monocytes express receptors for IL-4 on their cell surfaces. We demonstrate IL-4 acts to down regulate Interleukin-1 (IL-1) gene expression and secreted protein from lipopolysaccharide (LPS) stimulated monocytes. Human peripheral monocytes obtained from healthy volunteers were isolated on a Ficoll-Hypaque gradient and purified (> 95%) by adherence. Cells were cultured from 24 to 96 hours in serum-free medium in the presence of IL-4 with addition of LPS 10 µg/ml during the final 24 hr of incubation. Cell-free supernatants were assayed for IL-1 activity using the thymocyte mitogenic assay. IL-4 caused a dose dependent inhibition of IL-1 activity 45±13% (1000u/ml), 57±23% (200u/ml), 25±3.0% (100u/ml) and 0% (10u/ml) in 24 hr culture. Extending the incubation to 48 and 96 hr the effect of IL-4 was maintained (48 hr:200u/ml:23±1.0%, 20u/ml:3.0±1.0% and 96 hr, 200u/ml :24±1.0%, 20u/ml:20±1.0%. IL-4 has no effect alone or in combination with IL-1 on the bioassay. To determine the effect of IL-4 on IL-1β gene expression we cultured monocytes in the presence of LPS 10ug/ml and/or IL-4 (1000u/ml) and alone for 4 hr. Whole cellular RNA was isolated by the guanidinium-CsCl method. IL-1β mRNA was quantitated by dotting to nylon membranes and hybridizing to a P^{32} labeled cDNA probe. Densitometric scanning (relative densities compared to β-actin control) of the blots demonstrates IL-4 acts to down regulate IL-1 gene expression: LPS=32, IL-4=3.2, IL-4+LPS=12.7, no addition=6.9. These findings indicate IL-4 acts to suppress monocyte IL-1 production by altering gene expression.

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EXPRESSION OF CHONDROITIN SULFATES IN ACTIVATED RAT LIVER MACROPHAGES. A. Dokidis, C.R. Gardner, F.M. Robertson, D.L. Laskin and J.D. Laskin. UMDNJ-RW Johnson Medical School and Rutgers University, Piscataway, NJ 08854

Proteoglycans are extracellular glycoproteins important in such diverse macrophage functions as cell-cell recognition and adhesion. We have previously shown that lipopolysaccharide activated liver macrophages (LKC) incorporate inorganic sulfate into both

heparan and chondroitin sulfate proteoglycans. In the present studies we used specific monoclonal antibodies to characterize expression of different types of chondroitin sulfate in LKC. Three monoclonal antibodies were used which specifically recognize unsulfated (OS), 4-sulfated (4S) and 6-sulfated (6S) chondroitin sulfate. LKC were isolated from rat livers 24 hr following injection of 5 mg/kg LPS by pronase/collagenase and differential centrifugation. After 24 hr in culture, LKC were fixed in 70% ethanol and digested for 90 min at 37°C with 1 U/ml chondroitinase ABC. Cells were incubated with the antibodies for 30 min followed by FITC-conjugated secondary antibody. We found that LKC bind to all three anti-proteoglycan antibodies. Using fluorescence microscopy, OS, 4S and 6S chondroitin sulfate were, in general, uniformly distributed over the cell surface. Localized patches of fluorescence were observed on adherent cellular processes. By flow cytometry, a bimodel distribution of binding for each of the antibodies was observed. Approximately 40-50% of the activated LKC expressed OS and 4S chondroitin sulfate, while only 16% expressed 6S chondroitin sulfate. These results demonstrate that KC express chondroitin sulfates differing in side chain composition. Furthermore, these differences may be important in the functional responsiveness of activated liver macrophages. Supported by NIH GM34310.

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KINETICS AND REGULATION OF BOVINE ALVEOLAR MACROPHAGE PROCOAGULANT ACTIVITY Bruce D. Car, D. O. Slauson, M. M. Suyemoto, and N. R. Neilsen. Inflammation Research Laboratory, Pathology Department, College of Veterinary Medicine, Corneli University, Ithaca, New York, 14853

The surface expression of alveolar macrophage procoagulant activity (PCA), together with the ability to activate, and inhibit the activation of plasminogen combine to dictate the direction of pulmonary alveolar fibrin deposition or removal. Bovine pulmonary alveolar macrophages stimulated with *E. coli* endotoxin (LPS), *P. haemolytica* LPS and cytotoxin (crude preparation), and phorbol myristate acetate (PMA) show marked (5 - 15 fold) stimulation in PCA from basal levels, while dexamethasone lowers basal expression of PCA. *P. haemolytica* LPS appears more potent than *E. coli* LPS. Cytotoxin extract-induced activity occurs rapidly, at levels (1.7 activity units/ml) which in 8 hours incubation results in 100% cell death. Preincubation with dexamethasone (2.5×10^{-7} M) reduced PMA and LPS, but not cytotoxin extract induced PCA by 20 to 50%. Preincubation (1 hour) with actinomycin D (3 μg/ml) and cycloheximide (1 μg/ml) completely prevented the development of PCA induced by any mediator. Stimulation with LPS and PMA show slightly different kinetics of development, with the LPS-induced increase tending to occur earlier with the response saturating more quickly. Maximal PCA was completely removed by incubation with phospholipase A_2, phospholipase C, and was reduced 90% by incubation with Concanavalin A. Thus procoagulant activity was predominantly due to a tissue factor-like activity. This activity was 9 times more active with autologous bovine than with human plasma. (Supported in part by U.S.D.A. Grant Funds).

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ROLE OF EPSTEIN-BARR VIRUS GENE EXPRESSION IN HISTIOCYTIC ACTIVATION. Z. Dreyer, B. Dowell and K. McClain (Spon: D. Anderson). Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030

Infection-associated hemophagocytic syndrome (IAHS) is a poorly understood, frequently fatal illness which appears to represent the abnormal proliferation of hyperactivated hemophagocytic histiocytes throughout the lymphoreticular system. DNA hybridization to Southern blots have shown that Epstein-Barr virus (EBV) is frequently present at higher than normal levels in the lymphocytes of these patients. It is likely that the histiocytic proliferations are related either to the action of EBV gene products directly or to an aberrant immune response to the virus. In order to determine whether either or both of these hypotheses apply, we have performed in situ hybridization with ^{35}S-labeled DNA probes to determine the location of EBV DNA and RNA. In situ hybridization studies in 3 of 4 IAHS patients with prior confirmation of the presence of EBV by Southern blot have demonstrated dramatic hybridization patterns. Regions of tissue with very high levels of EBV gene expression have been identified, suggesting foci of infection. Also, there was evidence of diffuse EBV infection throughout the tissue which was clearly above control levels of the "background" that occurs when hybridizing with plasmid DNA. The levels of expression for several EBV genes as well as lymphokines and growth factors have been determined. Most of the hybridization signal was eliminated by pretreating tissue specimens with ribonuclease. Thus this is the first evidence of EBV gene expression in IAHS patients.

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THREE DIMENSIONAL STRUCTURE OF BEIGE MOUSE MACROPHAGE LYSOSOMES. P. Strausbauch and N. Sehgal. East Carolina University, Greenville, NC 27858-4354.

The three dimensional structure of beige mouse peritoneal macrophage lysosomes was ascertained by study of sequential electron micrographs of lysosomes taken in a series of serial sections. Multiple structural variants of macrophage lysosomes were observed which on two dimensional electron micrographs exhibit the appearance of elongated, dumbell-shaped, horseshoe-shaped and ring-shaped forms. There were also more complex, bizarre structures seen in many of the beige macrophage. The true configuration of these lysosomes became apparent after detailed three dimensional reconstructions. The elongated forms seen on electron micrographs represent long thin, serpentine structures. The dumbell-shaped forms represent a biconcave disc similar in structure to a red blood cell. The horseshoe-shaped and ring-shaped forms represent profiles taken through different planes of the same structure which consists of indention of a circular or oval disc to yield a three dimensional cup-shaped structure. The more bizarre forms of lysosomes represent the fusion of two or more of these types of lysosomes. Three dimensional reconstruction of these structures indicates that they can attain a large size and be composed of diverse structures - dumbells, horseshoes, rings and long extensions in which the basic structural unit appears to be fused biconcave discs and cup-shaped structures. A comparative study of lysosomes in animals not carrying the beige mutation reveals the presence of structurally analogous lysosomal variants in these animals. The presence of these peculiar lysosomes in macrophage from normal animals indicates that their presence in the beige animal is not a peculiar or abnormal finding unique to this animal but rather that their prominence in beige animals relates to their increased size and propensity to undergo fusion with similar structures. (Supported in part by NIH grant AI17162.)

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SURFACE DISTRIBUTION OF Fc RECEPTORS IN RAT ALVEOLAR MACROPHAGES ACTIVATED IN VIVO BY POLY I:C

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Alveolar macrophages express a variety of functional activities which allow them to play an important role in host defense in the lung. One of these activities is Fc mediated phagocytosis. We have shown previously that intratracheal (i.t.) administration of the interferon

inducer, poly I:C, leads to expression of enhanced functional activity in rat alveolar macrophages (Fed. Proc. 42: 6244, 1987). In an effort to better understand the nature of poly I:C activation of alveolar macrophages, we have studied the effect of i.t. poly I:C on several aspects of Fc receptor function. We have found that i.t. poly I:C enhances Fc mediated phagocytosis and rosetting of antibody coated RBC. It does not, however, affect the number or avidity of Fc receptors on macrophages as determined by Scatchard analysis of binding data for ^{125}I labelled soluble immune complexes. To determine if receptor topography may be involved in enhanced Fc receptor function, we examined the distribution of surface bound immune complexes on macrophages by electron microscopy with protein A - colloidal gold and a carbon-chromium replica technique. We found that the surface distribution of bound immune complexes on poly I:C activated macrophages was significantly different compared to alveolar macrophages from rats receiving i.t. saline. Our results suggest that the enhanced Fc receptor function of alveolar macrophages activated in situ by i.t. poly I:C, may be due, in part, to the topographic distribution or the mobility of Fc receptors within the membrane.

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RECEPTOR MEDIATED BINDING OF C-REACTIVE PROTEIN (CRP) TO HUMAN MONOCYTES AND THE U937 MONOCYTE CELL LINE OCCURS VIA RECEPTORS DISTINCT FROM IgG Fc-RECEPTORS. J.M. Tebo and R.F. Mortensen. The Ohio State University, Columbus, OH 43210.

Human C-reactive protein (CRP) is an acute phase reactant that is opsonic and an activator of macrophages (MØ) tumoricidal activity. CRP also activates the classical Complement pathway. These findings suggest that CRP may interact with MØ IgG Fc-receptors (FcR). Therefore, we characterized the specific binding of CRP to human blood monocytes and U937 cells. Specific saturable binding of ^{125}I-CRP to U937 cells occurred with a $K_d = 2 \times 10^{-7}$M. The number of binding sites per cell was calculated at 2×10^5. Polyclonal human IgG competitively inhibited ^{125}I-IgG binding. The human cell line K-562, which possesses FcRII and not FcRI, binds ^{125}I-CRP in a manner identical to that of U937 cells. The monoclonal Ab IV-3 which blocks IgG binding to FcRII, did not inhibit ^{125}I-CRP binding. CRP enhanced the binding of ^{125}I-labelled mAbIV-3 to U937 cells. The FcR mAb32 specific for FcRI did not prevent specific ^{125}I-CRP binding. Using the cross-linking reagent disuccinimdyl suberate (DSS), a single polypeptide membrane protein of 35-37 kDa was identified in detergent (CHAPS) extracts of U937 cells as a CRP-binding protein. The CRP-binding membrane protein has a size and cellular distribution that is distinct from that of IgG FcRI and FcRII. Thus, CRP binds to a membrane protein receptor that appears to be distinct from two of the human IgG FcRs; however, the CRP receptor may interact with the IgG FcRs. (Supported by USPHS grant CA 30015.)

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IMMUNOLOGIC MECHANISMS OF A TRAUMA ASSOCIATED GLYCOPEPTIDE A.N. Ozkan, S. Tompkins, S. Gregory, D.B. Hoyt Univ. of California Medical Center, San Diego, CA 92103 and Univ. of Chicago Medical Center, Chicago, IL 60637

Severe trauma results in the generation and release of a number of immunosuppressive factors. One of these factors, suppressor active peptide (SAP), is capable of profoundly inhibiting T cell blastogenesis as measured by the mixed lymphocyte reaction and mitogen activated responses. In vivo effects of SAP include the inhibition of cell-mediated immunity as assessed by a hypersensitivity assay utilizing dinitro-fluorobenzene. We have investigated the immunologic mechanisms behind these suppressive effects, specifically on inhibition of : 1) the expression of IL-2 receptors, 2) IL-2 biosynthesis, 3) calcium mobilization, and 4) calcium-calmodulin binding. The addition of suppressor peptide at concentrations producing 80% suppression of T cell blastogenesis (70 nM and 140 nM) had very little effect on IL-2 receptor expression on Con A activated peripheral blood mononuclear cells (PBMC). IL-2 biosynthesis at these concentrations was reduced by 60% and 78% respectively. Inhibitive activity of 100 nM SAP on IL-2 biosynthesis was partially reversed by the addition of 200 nM calcium ionophore A23187 (91% reduction in inhibition). Additional studies revealed that extracellular calcium influx in PHA activated PBMC was markedly inhibited (46% inhibition) by the addition of 70 nM SAP. Binding of calcium 45 isotope to immobilized calmodulin in the presence of SAP resulted in decreased binding of the isotope. The data presented here suggests that SAP-induced immunosuppression may in part be due to interference of calcium mobilization and calcium-calmodulin interactions leading to potential inhibition of key calcium-activated or calmodulin-dependent enzymes required for T cell activation and blastogenesis.

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CHARACTERIZATION OF MUCOSAL LYMPHOCYTES OF RABBIT GUT-ASSOCIATED LYMPHOID TISSUE. A.Ruiz , M.Roy and M.Gordon (Spon:C. Nacy). Walter Reed Army Institute of Research, Washington, D.C. 20307 and N.I.A.I.D., Bethesda, MD 20892

T-lymphocytes play important roles as regulatory and effector cells in mucosal immune responses. A monoclonal antibody, 93C6, was used to define a subpopulation of intestinal lymphocytes. In rabbit gut-associated lymphoid tissues (GALT), 93C6 antigen was expressed by lymphocytes in the domes and dome epithelium but not by cells from follicles of thymic-dependent regions. Immunoelectron microscopy revealed that this antigen was a surface membrane antigen on large(12um) and small(7um)lymphocytes. The 93C6-positive cells from GALT did not adhere to nylon wool, they lacked cytoplasmic granules or peroxidase activity, they expressed the rabbit T cell antigen 9AE10 and they proliferated in response to Concanavalin A (Con A) or Phytohemagglutinin (PHA). Immunohistochemical examination of rabbit tissues demonstrated 93C6-positive cells in the intestinal epithelium and lumen but they were rarely found in peripheral lymph nodes and they were not present in the thymus. When cultured for 48 hours with 10 ug ConA/ml, virtually all lymphocytes, whether from GALT or peripheral lymphoid tissues, expressed 93C6 antigen. ConA-stimulated thymocytes remained negative. Expression of this antigen appears to be correlated to the state of lymphocyte differentiation and histologic location. These studies demonstrate selective expression of a surface antigen by a subpopulation of mucosal lymphocytes. Further studies should delineate the roles that these cells play in mucosal immune responses and identify their functional significance.

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MIXED RBC-LEUKOCYTE AGGREGATES AND STABLE AUTOLOGOUS E-ROSETTES IN HUMAN BLOOD CULTURES STIMULATED BY TYPHUS GROUP RICKETTSIAE. T. Khavkin, M. Carl, M. Kuchler, M.J. Liao and D. Testa. Interferon Sci.,Inc. New Brunswick, NJ 08901 and Naval Med. Res. Inst. Bethesda MD 20814.

We have previously shown that 37°C stable autologous E-rosettes (SAR) and mixed RBC-leukocyte aggregates (MA) are formed in whole blood cultures upon activation of T lymphocytes with non-erythroagglutinating mitogens in the presence of neuraminidase (NASE) (10th Int. Histocomp. Conf. NY, 182, 1987). In the present study, MA and SAR, were elicited in whole blood cultures from donors seropositive to R. prowazekii or R. typhi, by incubation with a French Pressure cell sonicate of renografin-purified typhus group rickettsia or with lipopolysaccharide (LPS) of E. coli, in the presence of NASE. The fluorescent-phase contrast microscopy of acridine orange-stained

cultures revealed the lymphocyte-monocyte aggregates typical of cultures stimulated by mitogens. RBC consistently attached to aggregated lymphocytes, thus forming the MA. Solitary SAR formed around single lymphocytes were observed less frequently. The appearance of MA and SAR was associated with an increased incorporation of radioactive thymidine and production of γ-interferon, although overall expression of all these phenomena, especially SAR, was less extensive than those in parallel cultures exposed to the leukoagglutinating mitogenic lectin PHAL with NASE. None of these phenomena were observed in cultures from seronegative donors, and there were no SAR nor MA in seropositive cultures exposed to either rickettsial antigen, LPS or PHAL without NASE. The induction of MA and SAR in whole blood cultures by rickettsial antigens with NASE appears to be a simple qualitative assay for cell mediated immunity to typhus group rickettsiae.

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CHANGES IN LYMPHOCYTE SUBSETS IN PATIENTS SUFFERING FROM ACUTE THERMAL INJURY. F. Chrest, C. White, Y. Guo, W. Adler, A. Munster and R. Winchurch. Johns Hopkins Univ. Sch. Med. and Gerontology Res. Ctr. NIA, Baltimore, MD 21224.

The profiles of lymphocyte subsets in whole blood from patients suffering from burn injury were examined over a three week period post injury. Using two color flow cytometric analysis, changes in total T and B cells and discrete subsets were documented. In addition, those subsets expressing activation markers were identified. The data showed an early decrease in the absolute number and percent representation of T cells. Concomitantly, the percent of B cells was markedly increased, while absolute numbers were essentially unchanged. The early changes were most dramatic in severely burned patients. Simultaneous evaluation of $CD4^+$ ($Leu3^+$) and $CD8^+$ ($Leu2^+$) T cells revealed a significant decrease in the $CD4^+$ population while $CD8^+$ T cells were stable. The decline in $CD4^+$ cells was seen during the first 3 days following injury and was followed by a gradual increase in percent representation and absolute numbers as patients recovered. Two color analysis of the $CD4^+$ cell populations further indicated that the suppressor/inducer cells ($Leu3^+/Leu8^+$) rather than the helper ($Leu3^+/Leu8^-$) cells declined as a result of thermal injury. While levels of DR antigen on T cells remained unchanged, IL-2R expression increased during the first week following injury. The cells expressing IL-2R were of the $CD4^+$ and not the $CD8^+$ phenotype. Concomitant with these changes was an increase in soluble, cell-free IL-2R in the sera of burn patients. These increases were first evident 2-3 days after injury and then peaked 5-7 days later.

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MODULATION OF ANTIBODY RESPONSE BY IMMOBILIZATION STRESS. **W. ROSCOE, L. ROSS AND C.E. TAYLOR. THE MEDICAL COLLEGE OF PENNSYLVANIA, PHILA., PA.**

PREVIOUS WORK IN OUR LABORATORY HAS SHOWN THAT THE ANTIBODY RESPONSE OF ADULT SPRAGUE-DAWLEY RATS TO THE CAPSULAR POLYSACCHARIDE OF TYPE III STREPTOCOCCUS PNEUMONIAE (SSS-III) WAS SIGNIFICANTLY ($P<0.05$) LOWER IN 40D OLD RATS THAT HAVE BEEN SUBJECTED TO 2 WK OF NEONATAL IMMOBILIZATION (NI) STRESS. IN THE PRESENT STUDIES WE CONFIRMED OUR INITIAL FINDINGS THAT NI STRESS CAUSES SUPPRESSION OF ANTIBODY RESPONSE TO SSS-III, USING INBRED FISCHER 344 RATS. HERE, THE ANTIBODY RESPONSE OF THE STRESSED GROUP WAS ALSO LOWER THAN THAT OF A SECOND CONTROL GROUP OF ANIMALS, THAT WERE REMOVED FROM THE MOTHER (MATERNALLY DEPRIVED, MD) DURING THE STRESS PERIOD. IN ADDITION TO THE SSS-III ANTIGEN, STUDIES WERE CONDUCTED USING PSEUDOMONAS AERUGINOSA LIPOPOLYSACCHARIDE (PA). ALTHOUGH THE ANTIBODY RESPONSE TO PA WAS REDUCED IN THE STRESSED GROUP RELATIVE TO THE CONTROLS, THE DIFFERENCES WERE NOT STATISTICALLY SIGNIFICANT.

WE TESTED THE HYPOTHESIS THAT THE DECREASED ANTIBODY RESPONSE TO SSS-III WAS DUE TO AN INABILITY OF REGULATORY T AMPLIFIER CELLS TO PROLIFERATE IN RESPONSE TO THE ANTIGENIC STIMULUS, BY CONDUCTING A MITOGEN ASSAY USING CON A. OUR INITIAL RESULTS SHOW THAT THERE WERE NO SIGNIFICANT DIFFERENCES BETWEEN THE GROUPS. THUS, THESE STUDIES SUGGEST THAT THE DEGREE OF SUPPRESSION OD ANTIBODY RESPONSE BY IMMOBILIZATION STRESS DEPENDS ON THE ANTIGEN BEING TESTED, AND THAT THE SUPPRESSED ANTIBODY RESPONSE TO SSS-III IS NOT DUE TO AN IMPAIRMENT OF THE PROLIFERATIVE CAPACITY OF T LYMPHOCYTES.

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PRECURSOR DULL Ly-1 THYMOCYTES ALSO CONTAIN NK PRECURSORS. B.J. Mathieson, T. Gregorio, J. Wine and L. Mason (Spon: R. Wiltrout). Laboratory of Experimental Immunology, BRMP, NCI-FCRF, Frederick, MD 21701-1013.

Large granular lymphocytes (LGL) with natural killer (NK) activity, share several phenotypic and functional characteristics with T cell precursors and mature T cells. These similarities have led to speculation that NK cells might represent an immature form of T cells that have failed to differentiate into mature T cells because they have either encountered a defective environment, e.g., in the athymic mouse, or have failed to reach the appropriate environment for T cell differentiation and repertoire selection. We have investigated the lineage of NK cells by isolation and intrathymic transfer of donor, liver-derived NK cells into congenic, Ly-5-disparate hosts. The C57BL/6 Ly-5 congenic mouse pair permits detection of very small numbers of transferred cells (<1%, or 1×10^5) donor-derived cells). Following intrathymic transfer, of as few as 1×10^5 thymic precursors composed of the dull Ly-1 (low CD5, $CD4^-$,$CD8^-$ subset), differentiated thymocyte subsets can be generated. However, transfer of even 1×10^7 liver-derived LGL failed to generate thymocytes of donor origin up to 12 days after transfer. However, these LGL were able to adoptively transfer donor-derived LGL and NK activity into the liver. In contrast, dLy-1 cells that contain committed thymic precursors, and no detectable splenic colony forming units, were able to transfer LGL to the liver with i.v. transfer. Therefore, we have demonstrated that liver-derived LGL are not equivalent to thymic precursors and cannot give rise to T cells. However LGL can develop in the liver after i.v. transfer of a population of intrathymic cells containing an immature subset of pro-T cells. These data support the hypothesis of two independent lineages, possibly developing from a common precursor no longer capable of generating myelocytic subsets.

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DIFFERENTIAL EFFECTS OF PROTEIN KINASE C ACTIVATORS ON RAT LARGE GRANULAR LYMPHOCYTE (LGL) CHEMOTAXIS AND CYTOTOXICITY. A.M. Pilaro, T.J. Sayers* and R.H. Wiltrout (Spon: R. Wiltrout). Laboratory of Experimental Immunology, BRMP, and *BCDP, Program Resources Inc., NCI-FCRF, Frederick, MD 21701-1303.

We have been studying the effects of the protein kinase C (PKC) activators, 12-O-tetradecanoyl phorbol 13-acetate (TPA) and 1-oleoyl-2-acetyl glycerol (OAG) on several functions of isolated rat LGL. LGL were isolated from rat peripheral blood and enriched (> 80%) by

centrifugation over Percoll density gradients. Using the Boyden chamber technique, LGL displayed a dose and time-related chemotaxis to both OAG and TPA. The maximal effects were observed when LGL were incubated for 4 hr with 100 nM TPA or 100 uM OAG in the lower wells. Leukotriene B_4, complement fragments, N-formyl-methionyl-phenylalanine, and partially purified rat interferon α/β (IFN) also induced migration of LGL, although to a lesser degree. Chemotaxis was inhibited by pretreatment of the cells with the protein kinase C inhibitor, H-7. Conversely, HA-1004, which inhibits cyclic AMP-dependent protein kinases, did not inhibit LGL chemotaxis, suggesting that migration of these cells involves activation of protein kinase C. To determine whether activation of protein kinase C is also involved in LGL cytotoxicity, we conducted experiments to determine the ability of LGL to lyse the YAC-1 target, following stimulation by interleukin 2 (IL2) or IFN. Pretreatment of LGL with H-7 or HA-1004 had no effect on spontaneous or IL2 induced cytotoxicity, but H-7 did abrogate the ability of IFN to augment LGL-mediated cytolysis. These results suggest that IFN mediates the activation of LGL through a PKC-dependent pathway. Taken together, these results suggest that activated protein kinase C participates in the regulation of LGL chemotaxis and in the stimulation of cytotoxicity induced by IFN.

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AUGMENTATION OF MURINE SPLENIC NATURAL KILLER (NK) CELL ACTIVITY FOLLOWING SINGLE AND MULTIPLE INJECTION REGIMENS OF SOLUBLE GLUCAN. D. Williams, E. Jones, H. Pretus, R. McNamee and W. Browder. Departments of Physiology and Surgery, Tulane University School of Medicine, New Orleans, LA 70112.

Glucan, a soluble glucopyranose biologic response modifier, has been demonstrated to exert significant antineoplastic and immune stimulatory activity. Recently, we have shown that chronic glucan administration suppresses NK activity (Sherwood et al, J. Biol. Resp. Modif. 7:185, 1988). However, the temporal relationship between glucan administration and NK activity is unknown. The present study was undertaken to investigate the temporal relationship of single and multiple injection regimens of glucan on murine splenic NK cell activity. Male C57BL/6J mice were injected iv with glucan (200 mg/kg) on day 0. NK activity was assayed on days 1,3,6,9,12,15,21,28 and 35. Other groups of mice were injected IV with glucan (200 mg/kg) on days 0,3,6 and 9. NK activity was assayed on days 1,4,7,10,12,15,21 and 28. Dextrose (5% w/v) served as control. NK activity was evaluated at target:effector ratios of 1:50 and 1:100 employing the 4 hr YAC.1 ^{51}Cr release assay. A single injection of glucan increased NK activity by 63% ($p<0.005$) on day 1 and 44% ($p<0.001$) on day 3 at a T:E of 1:50. At a T:E of 1:100 NK activity was increased by 19% ($p<0.001$) on day 1 and 38% ($p<0.001$) on day 3. Interestingly, NK activity was suppressed by up to 33% ($p<0.001$) on day 28 following a single injection of glucan. Multiple glucan injections increased murine splenic NK activity on days 1 (19 to 63%) and 4 (17 to 20%) at T:E ratios of 1:50 and 1:100. However, by day 15 NK activity was suppressed by 38% ($p<0.001$) at 1:50 and 55% ($p<0.001$) at 1:100 in the multiple injection group. These data indicate that soluble glucan administration, in single or multiple injection regimens, will significantly enhance murine splenic NK activity at early time intervals. However, in agreement with previous reports, glucan will suppress NK activity at certain time intervals. These data extend the immunomodulatory effect of glucan by demonstrating augmentation of endogenous NK activity.

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The Effect of Acute *in vivo* Exposure to 4 ppm NO_2 on Murine Splenic T Lymphocyte Subpopulations. K. S. Damji and A. Richters. Univ. So. Cal. Sch. of Med., Dept. of Path., Los Angeles, CA 90033.

The effect of acute exposure to nitrogen dioxide (NO_2) on splenic T lymphocyte subpopulations was studied in C57BL/6cum mice. Ten mice were exposed in environmental chambers to 4ppm NO_2 for 8 hours, and ten mice served as filtered-air controls. Monoclonal antibodies to T lymphocyte differentiation antigens and fluorescence activated cell sorter (FACS) analysis were used to detect changes in T lymphocyte subpopulations. Percentages of T lymphocytes (Thy-1.2-positive), T-helper/inducer lymphocytes (L3T4-positive), and T-cytotoxic/supressor lymphocytes (Lyt-2-positive) were significantly lower ($p<0.05$) in NO_2-exposed animals compared to filtered air controls. Spleen and body weights of the mice were also determined and there were no differences in body weights of control and exposed animals, however, exposed mice had significantly lower spleen weights.

T lymphocytes play a central role in regulatory and effector immunological functions such as mediating delayed hypersensitivity, regulating immunoglobulin production, and lysing virus-infected and neoplastic cells. This is the first report providing evidence linking alterations in T lymphocyte subpopulations following exposure to a level of NO_2 encountered in industrial settings. Detection of alterations in T lymphocyte subpopulations using monoclonal antibodies and FACS analysis may provide an extremely sensitive means of demonstrating NO_2-induced changes in the immune system.

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ENDOTOXINS WITH DIFFERENT STRUCTURES BIND TO DISTINCT B LYMPHOCYTE GANGLIOSIDES. L. Brown, S. Vukajlovitch, D.C. Morrison, and J.L. Ryan (Spon: J.L. Ryan). VAMC and Yale Univ. School of Med., West Haven, CT. 06516. and Kansas Univ. Sch. of Med., Kansas City, Kansas 66101.

The molecular mechanisms by which bacterial endotoxins (LPS) activate immunocompetent cells are the subject of intense study. Membrane receptor complexes for LPS have not been clearly defined. We have used a radioiodinated photoactivatable, crosslinkable derivative of LPS to probe the membrane gangliosides to which LPS becomes intimately associated. Derivatives of smooth LPS (E.coli 0111:B4) and Rd LPS were prepared. B lymphocytes from the LPS-responsive C3H/HeN mouse were prepared by panning in 3% albumin and exposed to labeled LPS. The LPS was crosslinked to membrane using UV light. The procedure leaves a small iodinated ligand on the cell surface after LPS is removed by reduction. Membrane gangliosides were isolated by chloroform-methanol extraction, anion-exchange chromatography and Iatrobead chromatography. Gangliosides were displayed by two-dimensional thin layer chromatography (2D-TLC) and radioactivity on individual moieties was assayed by radioautography using hypersensitized film. We found that only a small percentage of the total ganglioside population is labeled with iodine under these conditions. While there are many similarities between the gangliosides labeled by the smooth and Rd forms of LPS, distinct differences were noted. Two gangliosides were labeled by the Rd LPS and not by the smooth LPS. Also there were two apparent density shifts suggesting that certain gangliosides were more accessible to one LPS compared to the other. These data suggest that different molecular forms of LPS interact with membrane gangliosides independently. Thus membrane gangliosides may play a role in triggering cellular responses to bacterial endotoxins.

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Synovial Tissue Macrophages In Human Rheumatoid Arthritis and Osteoarthritis. Shobha R. Chitneni, Karen L. Patton and J. Brice Weinberg. V.A. and Duke University Medical Centers, Durham, NC 27705.

Macrophages (MAC) are important mediators of tissue inflammation and cell growth. They can elaborate various products that may play a role in the pathogenesis of arthritis. These include hydrogen peroxide, superoxide, collagenase, and plasminogen activator, all of which can damage bone and cartilage. They can also produce prostaglandins, tumor necrosis factor, and interleukin-1 (IL-1) which can modulate the proliferation and function of synovial fibroblasts, as well as bone and cartilage cells. MAC-elaborated tissue factor (TF) may cause local clotting in the synovium and provide a provisional matrix for inflammatory cells. The purpose of this study was to evaluate the number and function of MAC in synovial tissue from humans with rheumatoid

arthritis (RA) and osteoarthritis (OA). Sterile surgical specimens from patients with OA (n=8) or RA (n=11) undergoing joint replacement for severe disease were processed by carefully and selectively removing the synovial tissue from the surgical specimen. The tissue was weighed, minced, incubated at 37°C in RPMI-1640 with collagenase (0.5 mg/ml) and trypsin (2.5 mg/ml) for 3 hours, passed through 20 um teflon mesh, and washed. Results are summarized:

	Wt (g)*	# SC*	SC/g	%NSE+*	%PO+	%MGC*	H_2O_2	IL-1	TF
OA	6±3	4±2	2±1	52±6	2±1	1±0	9±4	5	182
RA	14±3	51±17	4±2	69±6	3±2	4±1	25±17	9±6	358

(* signifies p<0.05 for OA vs RA)

Ninety five to 100% of the cells were viable (trypan blue exclusion). Synovial samples from RA patients were characterized by larger amounts of synovial tissue, more synovial cells (SC) (millions) and MAC [nonspecific esterase (NSE) + cells], and more multinucleated giant cells (MGC). The cytoplasm of the MAC, which were generally larger than blood monocytes, was vacuolated. As opposed to blood monocytes, few synovial MAC contained peroxidase (PO). In the unstimulated state, the MAC elaborated minimal amounts of hydrogen peroxide (nmol/hr/10^5 cells), but after treatment with 200 nM phorbol myristate acetate (PMA), MAC from patients with RA and OA elaborated significantly more. As determined by a mouse thymocyte proliferation assay, the MAC produced no or small amounts of interleukin-1 (IL-1) (ng/ml) in their basal state, but after culture with 1 ug/ml of endotoxin for 18 hours, they elaborated large amounts of this monokine. Freshly isolated synovial MAC displayed procoagulant activity (TF) (units/5x10^5 cells) as determined in a one stage, recalcification clotting test. Thus, MAC are present in synovial tissue and are capable of elaborating various inflammatory mediators. Furthermore, synovial tissue from RA patients contains more MAC than does that from OA patients. More studies of these cells may add to our understanding of the pathogenesis of arthritis.

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THE MECHANISM OF SPECIFIC UNRESPONSIVENESS TO RAT CARDIAC ALLOGRAFTS INDUCED BY B-LYMPHOCYTE PRETREATMENT.
Soji F. Oluwole, Tarik Wasfie, Mark A. Hardy

We have previously demonstrated that pretreatment with DST or spleen cells (SpL) induces specific unresponsiveness to rat cardiac allografts in the Lewis to ACI strain combination. This study examines the relative contribution of donor-B-lymphocytes (DBL) to the DST and SpL induced unresponsiveness. ACI recipients of Lewis cardiac allografts were pretreated with 2x10^7 DBL 7 days prior to transplantation. All pretreated recipients accepted their grafts permanently (>160 days). In the study of the in-vivo kinetics of DBL-induced donor-specific unresponsiveness by adoptive transfer of pooled sera or spleen T-lymphocyte subsets into naive ACI recipients of donor-type or third-party (WF) test cardiac allografts, transfer of 1ml serum on days 0 and 1 and 0.5ml on days +2, +3 and +4 relative to transplantation obtained from ungrafted, DBL-transfused rats led to specific prolongation of donor test graft from 10.6±1.1 days to 19.5±1.7 days (P<0.001). Similarly, serum from DBL-transfused and cardiac allografted recipients 30 & 100 days after transplantation increased the MST of donor test grafts in naive recipients to 31.3±2.5 days (P<0.001) & 20.0±2.5 days (P<0.001), respectively. Adoptive transfer of 2x10^7 T-lymphocytes from cardiac allograft recipients at 20 and 100 days into naive hosts 24 hours prior to transplantation led to significant donor-specific graft prolongation of 17.8±2.1 days (P<0.001) and 16.5±1.5days (P<0.01), respectively. Similarly, 0X8+ (CD8) T-cells obtained at 30 & 100 days after transplantation led to an MST of Lewis grafts of 16.3±0.9 days (P<0.001) & 16.5±1.2 days (P<0.01) respectively. Transfer of W3/25+ (CD4) T-cells in a similar manner failed to influence test graft survival. These results suggest that the immunosuppression effect of DST is, in part, mediated by B-lymphocytes & further demonstrate that the induction of unresponsiveness by DBL pretreatment is dependent on serum suppressor factor(s) while its maintenance is due to the interaction of serum suppressor factor(s) & 0X8+ (CD8) T-suppressor cells.

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TRANSFORMED FIBROBLAST, "FIBROHISTIOCYTOID CELL" INDUCTION IN-VITRO.
M.Takagi, M.Yamakawa, K.Tajima, S.Ohe, T.Osanai and Y.Imai. Yamagata Univ. Sch. Med., Yamagata, Japan, 990-23.

We have recently reported the existence of a specific cell type, called the "Fibrohistiocytoid cells(FH)", which were found in a variety of chronic inflammatory tissues. These "FH" include a series of cell types from a metamorphosized fibroblast(FB) to a certain cell type which resembles histiocytic FB: that is, their nuclei and cytoplasms have some similalities to FB and histiocyte, respectively. Moreover, they react immunohistochemically to lysozyme (Lyz), ferritin(Fer), α_1-antitrypsin(AT), α_1-antichymotrypsin(ACT) and Ia-like antigen(Ia). On the other hand, recent some authers have reported the functions of Ia+ FB in-vitro. We investigated Ia+ FB derived from human subcutis in-vitro to evaluate its morphology and functions in detail. Materials and Methods: FBs were obtained from healthy human subcutis and cultured as described previously (Mistui, 1987). The phenotypes of cultured FBs on the different population doubling levels(2-30PDL) were examined immunocytochemically and enzyme cytochemically. Adherent blood mononuclear cells(MNC) were examined as controls. Recombinant human interferon-γ (IFN-γ) was added into FB-cultured media as a Ia+ inducer. Results: IFN-γ treated FBs newly expressed Ia-like antigen(moderate HLA-DR+, partial and weak HLA-DP+ and -DQ+). No marked immunostains of Lyz, Fer, AT and ACT were found in early cultured FBs(2-4PDL). However, later cultured FBs(20-30PDL), although weaker than MNC, intensively reacted to the antisera. Enzyme activities of acid phosphatase, non-specific esterase and alkaline phosphatase were found in all PDL FBs. These immunostains and enzyme activities were independent of the addition of IFN-γ. Furthermore, no PDL FBs with or without IFN-γ expressed FcR, CR, immunophagocytosis and peroxidase activity. Conclusions: It suggests that cultured FBs under the defined in-vitro conditions express the analogous phenotypes to "FH". It is possible that "FH" are transformed FBs.

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MONOCYTE ACTIVATION IN AIDS RELATED COMPLEX (ARC).
C.-Spillert, F. Tecson-Tumang, J. Lombardo and E. Lazaro, U-MDNJ-New Jersey Medical School, Newark, NJ 07103-2757.

The necessity of activated monocytes in the development of AIDS and ARC is receiving great emphasis. The generation of monocyte procoagulant activity (MPA) by monocytes is a marker of this cell's activation. Since the monocyte is the only blood cell capable of generating MPA, the incubation of citrated blood with either saline (control) or endotoxin (monocyte activator) followed by determination of the recalcification time (RT) should yield a measure of monocyte activation. Citrated blood from 19 healthy volunteers (C), 22 with early ARC (EARC), 9 late ARC (LARC) and 7 with AIDS was incubated with 20 µl saline or 20 µl saline containing 10 µg endotoxin. After incubating at 37° for 2 hours (MPA in endotoxin sample) RT were measured. The mean RT saline and RT Endotoxin (min)±SD, HIV positive (%), percent abnormal RT values (outside range of control values) and significance are tabulated below:

GROUPS	RT SALINE	ABNORMAL %	RT ENDOTOXIN	ABNORMAL %	HIV+
C(n=19)	(A)6.6±0.8	0	(E)5.7±0.8	0	0
EARC(n=22)	(B)5.5±1.3	50	(F)4.1±0.8	73	41
LARC(n=9)	(C)4.7±1.2	89	(G)4.4±0.9	78	89
AIDS(n=7)	(D)5.1±1.3	86	(H)4.2±0.8	73	100

(A) VS (B)(C)(D)p<.001;differences between (B)(C)(D)p=NS
(E) VS (F)(G)(H)p<.001;differences between (E)(F)(G)p=NS

Conclusion: The above data obtained on hospitalized patients with EARC, LARC and AIDS indicates increased monocyte activation (decreased RT endotoxin). Reduced RT saline reflects a state of hypercoagulability. Whether shortened RT values can be an indicator for activation of the HIV virus in seropositive asymptomatic individuals remains to be determined.

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EVALUATION OF KUPFFER CELL Fc RECEPTOR FUNCTION IN VIVO AFTER INJURY. D.J. Loegering, F.A. Blumenstock, B.G. Cuddy. Dept. Physiology, Albany Medical College, Albany, NY 12208

Our previous studies have shown that Kupffer cell Fc receptor function is depressed after injury as determined using an in situ perfused rat liver system. Fc receptor function was depressed following thermal injury, endotoxemia and the phagocytosis of IgG-coated erythrocytes. The present study evaluated two different probes for assessing Kupffer cell Fc receptor function in vivo in rats. The first probe was aggregated IgG (AIgG). Jimenez and Mannik have suggested that small aggregates of IgG are an Fc receptor-specific probe. Heat aggregated IgG was fractionated and aggregates containing 3-10 IgG were used. AIgG were injected i.v. (0.1 mg/100g) and the tissue distribution at 10 min after injection was: liver, 23±0.7%; spleen, 0.9±0.1%, lungs, 1.8±0.1%; blood, 74±3%. The prior injection of large soluble aggregates of IgG (>10 IgG, 0.75 mg/100g) caused a 13% depression of the hepatic uptake of AIgG. The second receptor probe was IgG-coated erythrocytes (EIgG). EIgG were injected i.v. ($3x10^8$/100g) and hepatic uptake was determined 30 min after injection. Electron microscopy showed that the EIgG present in the liver had been phagocytized by Kupffer cells. The tissue distribution of EIgG was: liver, 78±1%, spleen, 9±1%, lungs, 0.5±0.1%, blood, 10±1%. The hepatic uptake of these receptor probes was used as an assessment of Kupffer cell Fc receptor function. The hepatic uptake of EIgG was depressed after thermal injury (under anesthesia) (20%, $p<.01$), endotoxemia (25%, $p<.001$) and the phagocytosis of IgG-coated erythrocytes (26%, $p<.001$), but the hepatic uptake of AIgG was unchanged in each case. These results indicate that EIgG may be an adequate in vivo probe for Fc receptor function, whereas AIgG is not. This study provides further evidence that Fc receptor function as assessed by the hepatic uptake of EIgG is depressed following injury, and that this impairment of receptor function may contribute to the depression of host defense function caused by injury. (GM-26102)

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A MORPHOLOGICAL, IMMUNOHISTOCHEMICAL AND ENZYME HISTOCHEMICAL STUDY OF INTRATHYROIDAL LYMPHOID FOLLICLES. Y. Imai, M. Yamakawa, K. Tajima, M. Takagi, S. Ohe and T. Osanai. Yamagata Univ. Sch. Med., Yamagata, Japan, 990-23.

Secondary lymphoid follicles(LFs) are found in many cases of autoimmune thyroid diseases including Hashimoto's thyroiditis. We have previously reported the existence and the importance of thyroglobulin-anti-thyroglobulin immune complexes in these germinal centers(GCs). However, it is still unknown the origin of follicular dendritic cell(FDC) as one of essential factors in GCs. We investigated the tissue samples from the patients with chronic thyroiditis to evaluate the formation and the development of intrathyroidal LFs. METHODS:Thyroid tissue samples were obtained by thyroidectomy. Twenty tissues with LFs were selected. The tissues were trimmed and immersed in 10%formalin, glutaraldehyde-osmic acid and periodate-lysine-paraformaldehyde(PLP) fixatives. A part of sliced tissues were snap frozen. The light and electron microscopic observations were routinely carried out. In addition, the immunohistochemistry and enzyme histochemistry were carried out on the PLP-fixed or fresh frozen cryostat sections. RESULTS and CONCLUSION:Primary and secondary LFs were found in chronic thyroiditis tissues. Moreover, a variety of cell clusters were found in the perivascular spaces. These cell clusters were composed of many lymphoid cells, a few macrophages and medium or large sized-fibroblast(FB)-like cells(Ia+, C3R-, S-100 protein-, DRC1-, AcPase+). Electron-microscopically FB-like cells had neither prominent pseudopodium nor labyrinthine structure. They had the intermediate characteristics between FB and macrophage: their nuclei and cytoplasms had some similarities to FB and macrophage, respectively, and then we have recently proposed the term "fibrohistiocytoid cells" for these FB-like cells. Moreover, occasional FB-like cells located in the perivascular areas without any cell clusters. In primary LFs, and the GCs and mantle zones of secondary LFs dendritic, DRC1+ cells were found. It suggests that FB-like cells in lymphoid cell clusters play as a trigger in the formation of LFs. Furthermore, we discuss the relationship between FB-like cell, and "dendritic cell" or FDC.

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FUNCTIONAL SIGNIFICANCE OF DECREASED SERUM ZINC IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) DISEASE. J. Falutz, C.M. Tsoukas, G. Deutsch (Spon. E. Skamene). Montreal General Hospital, McGill Univeristy, Montreal, Canada.

HIV infection causes progressive depletion of T helper (CD4) lymphocytes. The variable rate of decrease in CD4 cells suggests that co-factors may be important in disease progression. Zinc deficiency results in reversible abnormalities of cell-mediated immunity similar to those observed in HIV infection. We have found that serum zinc is decreased in advanced HIV disease. The relationship between nutritional status and immune parameters was studied in 27 HIV seropositive patients with variable clinical manifestations.

Serum zinc was determined by atomic absorption spectrophotometry. Serum albumin, a nutritional parameter, was measured by colorimetric techniques. T cell subsets were quantitated by flow cytometric analysis using monoclonal antibodies. Isolated peripheral blood mononuclear cells (PBMC) were cultured with phytohemagglutinin (PHA) and pokeweed mitogen (PWM) to which tritiated thymidine was added to determine incorporated radioactivity. Statistical analysis was based on Spearman's rank correlation coefficient.

A significant correlation was found between the zinc concentration (range 0.60-1.26 mg/L) and the proliferative response to PBMC to PHA ($p<0.01$) and PWM ($p<0.01$). No correlation was found between the absolute number of CD4 cells (range 16-765/microL) and the response to either PHA or PWM stimulation, regardless of clinical status. There was a significant correlation between serum zinc and albumin concentrations ($p<0.01$) as well as between the serum albumin levels (range 32-52 g/L) and the response to both PHA ($p<0.01$) and PWM ($p<0.05$) stimulation.

The lack of correlation between CD4 numbers and the response to mitogen stimulation is noteworthy. As most of our patients had normal albumin levels, the low serum zinc found was unlikely due to poor nutritional status. The decrease in serum zinc in patients with advanced HIV disease, may be an important cofactor in the progressive immunosuppression observed, as it is associated with low proliferative responses of PBMCs to mitogen stimulation.

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ANTIINFLAMMATORY EFFECTS OF NON-STEROIDAL ANTIINFLAMMATORY DRUGS (NSAIDs) INDEPENDENT OF AN EFFECT ON THE CYCLOOXYGENASE ENZYME. M. Forrest, V. Zammit and P. Brooks (Spon: G. Koo). Dept of Rheumatology, Royal North Shore Hospital, St. Leonards, 2065, NSW, Australia.

A property common to all NSAIDs is their ability to inhibit the cyclooxygenase enzyme of arachidonic acid metabolism. Whether or not this property is the sole determinant of their antiinflammatory efficacy remains contentious. We have addressed this problem by comparing the concentrations of 3 NSAIDs (indomethacin, ibuprofen and piroxicam) required for the inhibition of cyclooxygenase products, plasma leakage and neutrophil accumulation induced by endotoxin (10 ml; 500 ng/ml) in the rat subcutaneous air-pouch.

The injection of endotoxin into the air-pouch produced significant increases in plasma leakage, neutrophil accumulation and the cyclooxygenase products prostaglandin E_2, 6-oxo-prostaglandin F_{1a} and thromboxane B_2 but did not generate leukotriene B_4. All three NSAIDs produced dose related inhibition of these parameters. When IC_{50} values were compared, the

concentrations of NSAID required for inhibition of cyclooxygenase products were similar to those required for inhibition of plasma leakage but were significantly higher for inhibition of neutrophil accumulation. For example, the IC_{50}'s for inhibition of prostaglandin E_2, plasma leakage and neutrophil accumulation by piroxicam were 0.33 ± 0.21; 0.20 ± 0.29 and 23.39 ± 0.2 mg/kg respectively. These data indicate that while inhibition of prostaglandin production by NSAIDs may account for inhibition of plasma leakage, the inhibition of neutrophil accumulation is clearly independent of an effect on the cyclooxygenase enzyme.

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IMMUNIZATION AGAINST TRYPANOSOME CRUZI: ADJUVANTICITY OF GLUCAN. D. Williams, R. Yaeger, W. Browder, R. McNamee, E. Jones and H. Pretus. Departments of Physiology, Tropical Medicine and Surgery, Tulane University School of Medicine and School of Public Health and Tropical Medicine, New Orleans, LA 70112.

Trypanosome cruzi, the causative agent of Chagas disease, infects humans and animals who reside in tropical and subtropical regions. At present, there is no effective vaccine for T. cruzi. Glucan, a β-1,3 polyglucose biologic response modifier, possesses significant adjuvant activity. The present study investigated the adjuvant activity of glucan when combined with a vaccine of killed T. cruzi culture forms. ICR/HSD mice (20 g) were injected SQ with glutaraldehyde killed T. cruzi on days 21,14 and 7 prior to challenge with 50 T. cruzi blood forms. Glucan (1 mg/mouse) was administered either alone or in conjunction with T. cruzi vaccine. Isovolumetric dextrose served as control. Dextrose, glucan or T. cruzi vaccine as single treatment regimens showed 20.5, 21.4 and 21.6 day median survival times, respectively. In contrast, glucan administered with T. cruzi vaccine showed an 84.5% ($p<0.01$) survival at 275 days post-challenge. In addition, the number of T. cruzi observed in the blood of glucan-T. cruzi mice was lower than the appropriate controls. However, those animals which survived at 275 days were positive for the presence of T. cruzi by xenodiagnosis. Histopathologic evaluation revealed no parasites or cardiac pathology, but a mild splenic hyperplasia and inflammation of skeletal muscle was noted. Subsequently, mice were treated with the same regimen of glucan-T. cruzi and challenged with 500 or 5000 T. cruzi. Mice were monitored for survival and the presence of T. cruzi in the blood. Additionally, animals were sacrificed at the end of the study for histopathological evaluation. Glucan significantly ($p<0.05$) increased survival (60% and 50% glucan-T. cruzi vs 0% of control) and decreased parasitemia at both challenge doses. These data indicate that glucan will exert significant adjuvant activity when administered with killed T. cruzi. Additionally, glucan will exert significant protection over a wide challenge range.

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L-659,286, A SUBSTITUTED BETA-LACTAM, IS A SELECTIVE INHIBITOR OF EXTRACELLULAR HUMAN POLYMORPHONUCLEAR LEUKOCYTE ELASTASE. R.J. Bonney, A. Maycock, P. Dellea, K. Hand, D. Osinga, D. Fletcher, R. Mumford, J. Stolk, P. Davies and J. Doherty. Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.

L-659,286 (7α-methoxy-8-oxo-3-[[1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)thio]methyl]-5-thia-1-aza-6R-bicyclo[4.2.0]oct-2-ene-2-pyrrolidine carboxamide-5,5-dioxide) is representative of a large class of substituted β-lactams as a potent (K_i = 0.4 μM), time-dependent and selective inhibitor of human polymorphonuclear leukocyte elastase (PMN elastase). L-659,286 does not inhibit thrombin, trypsin, papain, plasmin, chymotrypsin, cathepsin G, blood clotting or the complement cascade at 50 μM. Since this compound inhibits tissue damage caused by the instillation of PMN elastase in the hamster, it has the potential for therapy of lung damage caused by the extracellular release of this enzyme (R.J. Bonney et al., J. Cellular Biochemistry, 1988, In Press). Therefore it was important to determine if such an inhibitor could inhibit PMN elastase located intracellularly where it has a critical role in killing and digesting infectious agents. PMN isolated from whole blood were incubated for 15 min with 21 μM L-659,286, a concentration well in excess of its IC_{50}. Subsequently an aliquot of cells were washed and degranulated with calcium ionophore A23187 or cytochalasin B plus formyl-methionyl-leucyl-phenylalanine. The release of elastase and myeloperoxidase was found to be the same in cells not exposed to inhibitor. Further experiments have shown that L-659,286 also inhibits PMN elastase mediated extracellular degradation of large molecular weight substrates. This indicates that this class of compounds has the potential of preventing degradation of connective tissue matrices in vivo without compromising vital intracellular functions of PMN elastase.

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IMMUNE STIMULATION FOLLOWING SINGLE OR MULTIPLE INJECTIONS OF SOLUBLE GLUCAN: TEMPORAL RELATIONSHIP. H. Pretus, R. McNamee, E. Jones, W. Browder and D. Williams. Departments of Physiology and Surgery, Tulane University School of Medicine, New Orleans, LA 70112.

Glucan, a soluble glucopyranose biologic response modifier, has been demonstrated to enhance diverse immunologic parameters. The present study was undertaken to evaluate the effect of single or multiple injection regimens of glucan on murine splenic macrophage tumoricidal activity, splenocyte mitogenesis and bone marrow proliferation as a function of time. Male C57Bl/6J mice were injected IV with glucan (200 mg/kg) on Day 0 and assayed on days 1,3,6,9,12, 15,21,28 and 35. Other groups of mice were injected IV with glucan (200 mg/kg) on days 0,3,6 and 9 and assayed on days 1,4,7,10,12,15,21 and 28. Dextrose (5% w/v) served as control. Glucan in the dose of 200 mg/kg has been shown to exert significant antineoplastic and antimicrobial activity. A single injection of soluble glucan increased macrophage tumoricidal activity against syngeneic melanoma B16 by 32% ($p<0.001$) on day 3, 19% ($p<0.02$) on day 6 and 39% ($p<0.01$) on day 9. Bone marrow proliferation showed a 45% ($p<0.0001$) increase on day 12, which continued to day 21. Glucan increased in vitro proliferation of splenocytes by 108% ($p<0.01$) on day 9 and the effect persisted to day 12. Splenocyte response to LPS showed a 381% ($p<0.0001$) increase on day 12 following a single injection of glucan. Multiple glucan injections increased macrophage tumoricidal activity by 40% ($p<0.01$) up to day 28. Bone marrow proliferation peaked (113%) on day 15 ($p<0.0001$) and continued for the duration of the study. In vitro proliferation of splenocytes and splenocyte response to LPS was significantly ($p<0.01$) elevated (89% and 289%, respectively) up to day 28 in the multiple injection group. These data indicate that glucan, administered in single or multiple injection regimens, will enhance macrophage tumoricidal activity, bone marrow proliferation, splenocyte proliferation and splenocyte response to mitogen for prolonged periods of time. However, multiple injections of soluble glucan resulted in even greater prolongation of immunologic stimulation, when compared to the single regimen.

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PHARAMACOLOGIC REGULATION OF THE DEVELOPMENT OF STREPTOCOCCAL CELL WALL INDUCED ARTHRITIS IN RATS. Jennifer Swisher, Janice Allen, Gerald Feldman, Larry Wahl and Sharon Wahl. NIH, Bethesda, MD 20892.

A single intraperitoneal injection of Group A streptococcal cell walls (SCW) induces a biphasic pattern of polyarthritis in susceptible rat strains. The first phase is an acute exudative response characterized by neutrophil accumulation and swelling which peaks at 3 days, recedes and is followed by a chronic destructive mononuclear cell-mediated phase. In order to define the cellular and molecular mechan-

isms responsible for these two distinct phases of the SCW-induced inflammatory response, we have evaluated the effect of site-specific inhibitors on these processes. Cyclosporin A (CsA), a specific inhibitor of T cell function, blocks the chronic mononuclear cell dependent phase of arthritis, but has no effect on the acute neutrophilic response. CsA inhibition of joint destruction is associated with decreased levels of interleukin-2, interleukin-3 and fibroblast growth activity. In contrast to CsA, the antiinflammatory corticosteroid, methylprednisolone (MP) ablates the acute response and consequently the infiltration of mononuclear cells. MP inhibition of arachidonic acid metabolism likely impairs SCW-induced enhancement of vasopermeability and leukocyte chemotaxis which is manifested as lack of joint swelling and leukocyte infiltration (articular index (AI) 9.1 vs 0.2). By comparison, flurbiprofen, a nonsteroidal antiinflammatory drug, partially suppressed both the acute and chronic arthritis (AI 9.1 vs 2.1). Thus, each of these agents appears to act at different loci to modulate SCW-induced arthritis. By choosing drug combinations with different target specificities, it may be possible to interrupt several sites in this interdependent inflammatory process. Such combinations may allow the use of lower drug doses with improved efficacy and reduced toxicity.

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CELLULAR IMMUNITY IN MULTIPLE MYELOMA (MM): MODULATION BY RANITIDINE. H. Nielsen, H.J. Nielsen, K. Klarlund, A. Drivsholm, F. Moesgaard, H. Kehlet. Statens Seruminstitut, Hvidovre hospital and Rigshospitalet, Copenhagen, Denmark.

Ranitidine, a histamine antagonist, has shown immunostimulatory properties in human models of defective cellular immunity. Since MM is characterized by various immune defects we studied eleven patients with untreated MM in a randomized double-blind trial of ranitidine 600 mg daily for three weeks (7 patients ranitidine and 4 patients placebo). Skin test (seven antigens), NK cell activity and monocyte chemotaxis and oxidative metabolism were measured days 0 and 22. Generation of superoxide anion upon stimulation with PMA 50 ng/ml was significantly enhanced after ranitidine treatment (3.9 vs. 2.5 nmol/min/10^6 monocytes, P = 0.04). Chemotaxis to C5a and fMLP was depressed before treatment and unchanged by ranitidine. NK cell activity was defective (18 % lysis) and not affected by treatment (17 % lysis). However, IL-2 stimulated NK cell activity (33 % lysis pretreatment) was lower after ranitidine (28 % lysis, P = 0.02) as were interferon stimulated NK cell activity. Skin test was not significantly altered by treatment. Patients given placebo showed no difference in immune functions. Routine laboratory analysis inclusive immunoglobulins were not altered during treatment with ranitidine.

In conclusion, the ability of monocytes to release toxic oxygen radicals was significantly enhanced by ranitidine, which may reflect the possible role of histamine in the regulation of monocyte oxidative metabolism. Further, stimulated NK cell activity was diminished after treatment, while monocyte chemotaxis, spontaneous NK cell activity and delayed hypersensitivity in the skin were not altered by histamine blockade in patients with MM.

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HETEROGENEITY OF LYMPHOCYTE CYCLOSPORINE A BINDING SITES AND THEIR EFFECT ON MEMBRANE FLUIDITY. C.D. Niebylski and H.R. Petty. Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

Cyclosporine A (CsA), is an immunosuppressive agent of clinical utility. Its mechanism of action is unknown. This study characterizes cellular CsA binding sites in living peripheral blood lymphocytes (PBLs) and their influence on membrane fluidity. Light scattering and dye intercalation experiments showed that CsA's critical micelle concentration is 3μM. All experiments were conducted below 3μM to avoid artifactual label and lipid partitioning. Fluorescence microscopy of PBLs labeled with N-dansyl-D-lysyl-8-cyclosporine (dans-CsA) revealed binding sites associated with plasma membranes and intracellular granules. Fluorescence lifetime and emission spectra showed two distinct dans-CsA environments differing in hydrophobicity. Fluorescence depolarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) was employed to measure PBL membrane fluidity. Fluorescence microscopy showed that DPH is found in plasma and intracellular membranes. At 37°C PBL exhibited a rapid and marked increase in fluidity at 10^{-7}M CsA. Polarization (P) of DPH emission decreased to P=.150±.010 from .210±.015 for controls (n=5). Kinetic studies showed that membrane fluidization was complete 30 min. after the addition 10^{-7}M CsA at 37°C. The decrease in membrane order was also studied using time resolved-fluorescence anisotropy to measure the rotational correlation time of DPH. At 37°C this time decreased from control values of 5.7±0.3 nsec to 2.3±0.2 nsec (n=3) for PBLs incubated with 10^{-6}M CsA. The limiting anisotropy dramatically decreased from controls (.110±.010) to 10^{-6}M CsA-treated cells (.030±.005). PBLs show a dramatic increase in membrane fluidity at physiologically relevant doses. This suggests that an early event in CsA's activity is membrane binding followed by an increase in membrane fluidity.

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ARACHIDONIC ACID METABOLISM IN CAPRINE ALVEOLAR MACROPHAGES. M.D. Englen, S.M. Taylor, W.W. Laegreid, R.M. Silflow, K.L. Banks and R.W. Leid. Dept. of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040.

Although the importance of arachidonic acid (AA) metabolites as mediators of pulmonary inflammation is well established, little information is available on AA metabolism by alveolar macrophages (AM) of most domestic animals. We examined the in vitro release of AA metabolites from caprine AM (CAM) stimulated with the calcium ionophore A23187 or opsonized zymosan (ZYM). CAM were collected from the lungs of normal adult goats by bronchoalveolar lavage. CAM were adhered to 35mm culture wells for 1 hr at 37°C in RPMI-1640, washed, then labelled for 1 hr with 5μCi ^{3}H-AA. Unincorporated ^{3}H-AA was removed and A23187 (10μM) or ZYM (1 mg) was added to the CAM cultures. After 4 hr incubation at 37°C, AA metabolites were extracted from CAM culture medium by chloroform/ethanol. AA metabolites released by CAM were analyzed by reverse-phase HPLC with UV detection and on line radiometry:

^{3}H-AA Metabolites (DPM/10^6 cells ±SEM, n=5)

	A23187	ZYM
TXB_2	ND[1]	328±223
$PGF_{2\alpha}$	ND	468±261
PGE_2	ND	656±249
HHT	971±589	2,143±540
LTB_4	7,781±1,245	3,695±245
5-HETE	4,265±2,667	3,263±1,971
12-HETE	1,276±560	1,216±665
AA	41,216±16,122	33,617±7,831

1 Not Detectable. No AA metabolites were detected from unstimulated cAM control cultures. Our work is the first demonstration of AA metabolism by CAM and indicates a potential involvement of AA metabolites in mediating caprine pulmonary inflammatory events.

Supported by grants from the Washington Technology Center and the Agricultural Research Center, Wash. St. Univ.

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LYMPHOKINE - ACTIVATED KILLER (LAK) CELLS DISCRIMINATE BETWEEN SMALL ALLOGENEIC AND SYNGENEIC LYMPHOCYTES IN VITRO. B. Rolstad, J.T. Vaage and S. Fossum Anatomical Institute, University of Oslo, Karl Johans gate 47, N-0162 Oslo 1, Norway.

Lymphokine activated killer cells (LAK cells), i.e. lymphocytes activated in vitro by treatment with Interleukin 2 (IL-2), apparently have a unique capability of recognizing and killing malignantly transformed cells, whereas they usually spare normal, differentiated cells. They have therefore attracted much interest as putative effector cells in the surveillance against cancer. The target cell specificities and the effector cell recognition structures involved in conjugation and lysis are as yet unknown. MHC gene products are believed not to be the targets for LAK cells, because differentiated cells, expressing MHC gene products, whether syngeneic or allogeneic to the LAK cells, are usually poor targets. Here, we demonstrate that small allogeneic lymphocytes are susceptible to killing by LAK cells in vitro in certain strain combinations of rats, provided that phytohemagglutinin (PHA) is present during the cytotoxic assay. The mechanisms of LAK cell recognition of allogeneic lymphocytes must be sought within major histocompatibility complex (MHC) gene products, because a major histocompatibility difference between effector and target cells by itself was sufficient to elicit target cell destruction. Furthermore, the killing did not involve post-thymic cytotoxic T cells, since increased killing of allogeneic lymphocytes was observed when the LAK cells were generated from athymic nude rats instead of from normal euthymic rats.

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NEW PERSPECTIVES ON THE ACUTE INFLAMMATORY PROCESS, GAINED FROM ANALYSES OF EXTRACELLULAR FLUIDS (OBTAINED BY ORGAN CULTURE). A. M. Dannenberg, Jr. Johns Hopkins School of Hygiene and Public Health, Baltimore, MD 21205.

The roles of various serum components and leukocytes were assessed in developing and healing inflammatory skin lesions. The lesions were produced in rabbits by the topical application of 1% sulfur mustard. They were removed from the pelt and organ-cultured for 24 hr. The culture fluids extracted (from the extracellular fluids within the lesions) factors that modulated the inflammatory response. The type and degree of activation of cells within the lesions were determined both histologically and histochemically. In peak lesions, extravasated serum was not static, but turned over 3 times each day. Its protease inhibitors (α_1-proteinase inhibitor and the α-macroglobulins) were found to be major factors controlling the damage produced by extracellular proteases. The levels of these proteases were highest in healing lesions. PMN appeared to pass through the lesions en route to the surface, where they died and became a major part of the lesion crust. The crust, though inspissated, contained active lysosomal enzymes. Within the lesion proper, activated macrophages and activated fibroblasts, but not PMN, were major sources of extracellular lysosomal enzymes. During all stages of lesion development and healing, extracellular chemotactic activity for both PMN and macrophages was high. These results indicate (a) that extravasated serum (which is known to contain antioxidants as well as protease inhibitors) is a beneficial (rather than detrimental) modulator of the inflammatory process; (b) that macrophages and fibroblasts are often the major participants of the acute, as well as the chronic, inflammatory process; and (c) that crusts are not just a passive protective barrier, but contain active enzymes (and perhaps growth factors) that leach out of the moist crust base and participate in the healing process. (Supported by U.S. Army Contract No. DAMD17-80-C-0102.)

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Kininogenases Kallikrein 7: Physiological Properties and Pharmacological Rationale—Experimental Studies

G.L. Haberland, J.W. Rohen, and H. Fritz, Editors

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