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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

See attached Summary of Symposia

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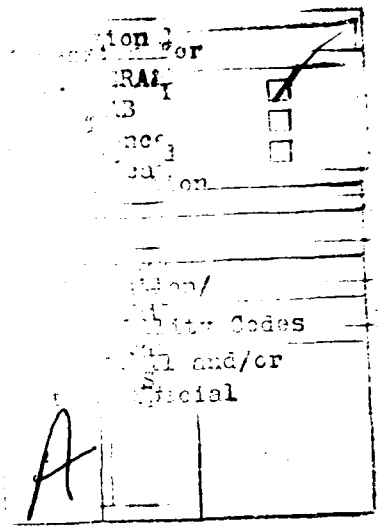

SYMPOSIA SUMMARY
19th Annual Meeting Reticuloendothelial Society
Chase Park Plaza Hotel, St. Louis, Missouri

SYMPOSIUM A: Chaired and Introduced by Dr. Steven D. Douglas

Keynote Address:

Dr. Ira Pastan
National Institutes of Health
"Role of the Receptosome in Receptor-Mediated Endocytosis".

Studies related to the mechanisms whereby receptor ligands complexes are transmitted from the plasma membrane through coated pits, coated vesicles, and receptosomes to the Golgi region of the cell were reviewed. These studies are a fundemental importance in macromolecular transport in diverse systems.

Dr. Philip Stahl, Washington University School of Medicine, St. Louis, MO.
"Modulation of the Mannose Specific Endocytosis Receptor of Macrophages".

The receptor system for fucose mannose BSA was discussed. The characteristics of this receptor and its inhibition were delineated. This receptor is important in the fate of lysosomal enzymes, opsonization, and perhaps macrophage-lymphocyte interaction.

Dr. Elliot Schiffman, National Institutes of Health, Bethesda, MD.
"Receptors for Chemotactic Peptides".

Recent novel observations on the regulation of leukocyte chemotaxis were presented and basic mechanisms of neutrophil and macrophage adherence and motility were reviewed.

Dr. Isiah Fidler, recipient of the Maria T. Bonazinga Award of the Accurate Chemical Company, discussed macrophage mechanisms of tumoricidal activity at the RES Banquet. These mechanisms are important in host response to tumor systems. Mechanisms for activating macrophages with lymphokines and liposomes were considered.

SYMPOSIUM B: Chaired by Dr. Philip Davies

Dr. Daniel Steinberg, University of California San Diego, La Jolla, CA.
"Metabolism of Lipoproteins by Monocyte-Macrohpages".

Receptors for low density lipoproteins in relationship to accumulation of lipids by macrophages and athregenesis were considered.

Dr. Robert Bonney, Merck Institute Therapeutic Research, Rahway, NJ
"Regulation of Prostaglandin and Leukotriene Production by Macrophages".

The modulation of macrophage function by prostaglandins and leukotrienes was illustrated.

Dr. Peter Henson, National Jewish Hospital, Denver, CO
"Release of Platelet Activating Factor and other Lipid derived Macrophages".

The mast cell, neutrophil and monocyte-derived platelet activating factor has been characterized and its effects on various cell types described.

Dr. T. Suzuki, University of Kansas Medical Center, KC
"Fc Mediated Receptor Modulated Regulation of Macrophages Fc Receptor Functions".

Receptors for IgG_{2A} and IgG_{2B} are described and characterized.

SYMPOSIUM C: Chaired by Dr. John K. Spitznagel

Dr. Lawrence DeChatelet Memorial Symposium

Dr. Emil Skamene, McGill University, Montreal, Quebec, Canada
"Genetic Analysis as a Probe of Macrophage Microbicidal Responses".

Discussed genetic control of macrophage response to microorganisms and parasites.

Dr. Pamela Shirley, Bowman Gray School of Medicine, Winston-Salem, NC
"Oxidative Bactericidal Mechanisms in Neutrophils".

Discussed oxidative mechanisms for bactericidal activity.

Dr. John Spitznagel, Emory University, Atlanta, GA
"Non-oxidative Bactericidal Mechanisms in Neutrophils".

Reviewed non-oxidative microbicidal mechanisms.

Dr. Peter Elsbach, New York University, NY
"Membrane Active Bactericidal Protein from Human and Rabbit Neutrophils".

Described a new bacterial bactericidal permeability increasing protein which is important in killing bacteria.

Dr. David Bass, Bowman Gray School of Medicine, Winston-Salem, NC
"Microbicidal Mechanisms of Eosinophils".

Considered eosinophil microbicidal mechanisms.

Dr. Paul Quie, University of Minnesota, Minneapolis, MN
"Clinical Syndromes with Impaired Microbicidal Function".

Presented clinical disorders related to defects in neutrophil and monocyte microbicidal function.

RES

Program and Abstracts

OF PAPERS FOR
THE NINETEENTH NATIONAL MEETING
OF THE RETICULOENDOTHELIAL SOCIETY

OCTOBER 17-20 CHASE PARK PLAZA HOTEL
ST. LOUIS, MISSOURI

COVER: GATEWAY ARCH

Overlooking the mighty Mississippi is the nation's tallest monument, the 630 foot Gateway Arch. The Arch serves as a gleaming tribute to the early pioneers and the westward expansion. The observation deck, with its sixteen large windows, offers magnificent views to the east and west of the Arch.

The Museum of Westward Expansion is located in the underground chamber beneath the Arch. The exhibit focuses on a span of time in the 1880's and is magnificently told in the cryptic words of those who made history - people like Lewis and Clark, Thomas Jefferson, and the many unnamed settlers, miners, farmers and soldiers, who battled the wilderness. The Museum and the Arch are open year-round.

NINETEENTH ANNUAL NATIONAL MEETING
RETICULOENDOTHELIAL SOCIETY

October 17-20, 1982
Chase Park Plaza Hotel
St. Louis, Missouri

The Reticuloendothelial Society acknowledges with sincere gratitude the financial support provided by the following:

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The Reticuloendothelial Society also acknowledges the continuing financial support provided by our Corporate Members for 1982.

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The Scientific Program Committee wishes to express its sincere appreciation to the staff, faculty and administration of Washington University and St. Louis University for their help in arranging this meeting.

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Charles D. Alley
Dien-Ming Chen
Carlos Daughaday
Hsiu-San Lin
Carl W. Pierce
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Philip Davies
John K. Spitznagel

SYMPOSIUM SPEAKERS

Paul J. Edelson
Philip Stahl
Elliot Schiffman
Daniel Steinberg
John Humes
Peter Henson
Tsueno Suzuki
Emil Skamene
Lawrence R. DeChatelet
John K. Spitznagel
Peter Elsbach
David A. Bass
Paul G. Quie

KEYNOTE SPEAKER

Ira Pastan

COMMITTEE ON PRESIDENTIAL AWARDS

Sigurd Normann, Chair
Robert Evans
Peter Abramoff

WORKSHOP

October 16 and 17, 1982 — Washington University, School of Medicine

RETICULOENDOTHELIAL CELL FUNCTION

Organizing Committee:
Herbert B. Herscowitz, Chair
Philip Stahl, Local Host
James W. Scheffel
David Clark

Saturday, October 16, 1982 8:30 a.m.- 5:00 p.m.

GENERAL SESSION ON MACROPHAGES

Collection and Cytochemical Identification of Macrophages
Dr. Carleton C. Stewart - Los Alamos National Laboratory

Enrichment and Depletion of Macrophages
Dr. Luigi Varesio - National Cancer Institute

Identification of Macrophage Surface Receptors
Dr. John Atkinson - Washington University Medical School

or

Saturday, October 16, 1982

Session 1: 8:30 a.m.-12:00 noon

Receptor-Mediated Endocytosis
Dr. Marilyn Konish and Dr. Virginia Shepherd - Washington Univ. Medical School

Chemotaxis Assay
Drs. Robert M. Senior and Gail C. Griffin - Jewish Hospital of St. Louis

Macrophage Growth Factor Production and Assay
Dr. Dien-Ming Chen and Dr. H. Lin - Washington University Medical School

Macrophage-Mediated Bone Resorption
Dr. Arnold Kahn and Dr. Steven Teitelbaum - Washington University Medical School

Session 2: 1:00 p.m.-5:00 p.m.

Quantitation of Surface Markers and RE Cell Function by Fluorescence Microscopy
Dr. James Wedner - Washington University Medical School

Preparation of Monoclonal Antibodies - Hybridoma Technology
Dr. Brian Clevinger - Washington University Dental School

Natural Killer (NK) Cell Cytotoxicity Assay
Dr. Edwin W. Ades and N. Ann Hinson - Lilly Research Laboratories

Separation of Macrophage Subpopulations by Density Gradient Centrifugation
Dr. Abdul Ghaffar - University of South Carolina Medical School

Sunday, October 17, 1982 8:30 a.m.- 12:00 noon

In Vitro and In Vivo Phagocytosis
Dr. Abdul Ghaffar - University of South Carolina Medical School

Microbicidal Acitvity of Macrophages
Dr. Russell Little - Jewish Hospital of St. Louis

Detection of Macrophage Enzymes
Dr. Edward Campbell - Jewish Hospital of St. Louis

Macrophage Tumor Cell Killing
Dr. Robert H. Wiltrout - National Cancer Institute

Use of Macrophage Tumor Cell Lines as Models of Macrophage Function
Dr. Howard T. Holden - National Cancer Institute

PROGRAM

Sunday, October 17, 1982

8:30 a.m.-1:00 p.m. — RES COUNCIL MEETING — Georgian Room

12:00-7:30 p.m. — REGISTRATION — Chase Park Plaza Hotel, Chase Lounge

2:00-5:00 p.m. — Chase Club
WORKSHOP - "Nomenclature of Natural Killer and Natural Cytotoxic Cells"
Dr. Ronald B. Herberman, NCI, NIH, Chair. Participation in this "dry" workshop is open to all individuals interested in natural cell mediated cytotoxicity. Individuals who have worked with NK cells, particularly heterogeneity of effector cells are invited to bring 1 or 2 slides to illustrate their work.

7:00 p.m. — OPENING PROGRAM — Chase Club

GREETINGS
Dr. John K. Spitznagel, President

WELCOME TO ST. LOUIS
Thomas R. Fitzgerald, S.J. President, St. Louis University
William Danforth, M.D., Chancellor, Washington University

GREETINGS AND ANNOUNCEMENTS
Dr. David Thomasson, Chair, Local Host Committee

7:30 p.m. — Chase Club
Introduction of the Keynote Speaker - Dr. Steven D. Douglas, Chair, Scientific Program Committee.

KEYNOTE ADDRESS
Dr. Ira Pastan, Chief, Laboratory of Molecular Biology, NCI, NIH
"Role of the Receptosome in Receptor-Mediated Endocytosis"*
*Studies in collaboration with Dr. Mark Willingham

8:30 p.m. — Chase Club
SYMPOSIUM A — "Mononuclear Phagocyte Plasma Membranes & Receptors"

10:00-11:30 p.m. — MIXER — Regency Room

Monday, October 18, 1982

8:00 a.m.-5:00 p.m. — REGISTRATION — Chase Lounge

8:30 a.m.-5:00 p.m. — EXHIBITS — Khorassan A

8:30-10:30 a.m.
TARGETED SESSION #1 - Stockholm Room - "Macrophages & the Immune Response", Papers 1-7.
TARGETED SESSION #2 - Empire Room - "Regulation of Inflammation", Papers 8-14.
TARGETED SESSION #3 - Georgian Room - "Pulmonary Defense Mechanisms", Papers 15-20.

10:15 a.m. — COFFEE BREAK — Khorassan A

10:30 a.m.-12:15 p.m. — Khorassan A
POSTER SESSION I - Papers 21-29
EXHIBITS

12:30-2:00 p.m. — SELECTED TOPICS LUNCHEON — Mr. Sam's

2:00-5:00 p.m. — Chase Club
SYMPOSIUM B — "Lipid Related Secretory Functions of Mononuclear Phagocytes"

5:00-6:00 p.m. — Business Meeting — Chase Club

6:30-8:30 p.m. — Reception — Tiara Lounge

Tuesday, October 19, 1982

8:30 a.m.-5:00 p.m. — EXHIBITS — Khorassan A

8:30-10:30 a.m.
TARGETED SESSION #4 - Stockholm Room - "Cytotoxicity: Macrophage & Natural Killer Cells", Papers 30-36,84.
TARGETED SESSION #5 - Empire Room - "Regulation of Macrophage Function and Secretion", Papers 37-42.
TARGETED SESSION #6 - Georgian Room - "Antimicrobial Functions", Papers 43-48.

10:15 a.m. — COFFEE BREAK — Khorassan A

11:00 a.m.-12:30 p.m. — Khorassan A
POSTER SESSION II - Papers 49-57, 85, 87, 89
EXHIBITS

1:30-3:45 p.m.
TARGETED SESSION #7 - Stockholm Room - "Host Response to Tumors", Papers 58-64.
TARGETED SESSION #8 - Empire Room - "Macrophage Differentiation & Proliferation", Papers 65-71, 86.

3:30 p.m. — COFFEE BREAK — Khorassan A

3:45-5:30 p.m.
TARGETED SESSION #9 - Stockholm Room - "Macrophage Heterogeneity", Papers 72-77.
TARGETED SESSION #10 - Empire Room - "Neutrophil Function", Papers 78-83, 88.

7:00-9:00 p.m.
RECEPTION - Zodiac Room
SOCIETY BANQUET; AWARDS - Starlight Room

Wednesday, October 20, 1982

8:00 a.m.-12:00 p.m. — Chase Club
Lawrence R. DeChatelet Memorial Symposium — "Phagocyte Microbicidal Mechanisms"

10:10 a.m. — COFFEE BREAK — Regency Room

SYMPOSIUM A

Sunday, October 17th
Chase Club

MONONUCLEAR PHAGOCYTE PLASMA MEMBRANES & RECEPTORS

Paul J. Edelson presiding

8:30 p.m.
Paul J. Edelson, Harvard Medical School, Boston, MA
ECTOENZYMES

9:00 p.m.
Philip Stahl, Washington University School of Medicine, St. Louis, MO
MODULATION OF THE MANNOSE SPECIFIC ENDOCYTOSIS RECEPTOR OF MACROPHAGES

9:30 p.m.
Elliot Schiffman, NIH, Bethesda, MD
RECEPTORS FOR CHEMOTACTIC PEPTIDES

SYMPOSIUM B

Monday, October 18th
Chase Club

LIPID RELATED SECRETORY FUNCTIONS OF MONONUCLEAR PHAGOCYTES

Philip Davies presiding

2:00 p.m. INTRODUCTION

2:10 p.m.
Daniel Steinberg, Univ. of California San Diego, La Jolla, CA
METABOLISM OF LIPOPROTEINS BY MONOCYTE-MACROPHAGES

2:40 p.m.
John Humes, Merck Institute Therapeutic Research, Rahway, NJ
REGULATION OF PROSTAGLANDIN AND LEUKOTRIENE PRODUCTION BY MACROPHAGES

3:10 p.m. Coffee Break

3:30 p.m.
Peter Henson, National Jewish Hospital, Denver, CO
RELEASE OF PAF AND OTHER LIPID DERIVED MEDIATORS

4:00 p.m.
Tsueno Suzuki, Univ. of Kansas Medical Center, Kansas City, KS
Fc RECEPTOR MEDIATED REGULATION OF MACROPHAGE FUNCTION

4:30 p.m. GENERAL DISCUSSION

LAWRENCE R. DeCHATELET MEMORIAL SYMPOSIUM

Wednesday, October 20th
Chase Club

PHAGOCYTE MICROBICIDAL MECHANISMS

John K. Spitznagel presiding

8:30 a.m. INTRODUCTION

8:40 a.m.
Emil Skamene, McGill University, Montreal, Quebec, Canada
GENETIC ANALYSIS AS A PROBE OF MACROPHAGE MICROBICIDAL RESPONSES

9:10 a.m.
Lawrence R. DeChatelet, Bowman Gray School of Medicine, Winston-Salem, NC *
OXIDATIVE BACTERICIDAL MECHANISMS IN NEUTROPHILS

9:40 a.m.
John K. Spitznagel, Emory University, Atlanta, GA
NON-OXIDATIVE BACTERICIDAL MECHANISMS IN NEUTROPHILS

10:10 a.m. Coffee Break

10:30 a.m.
Dr. Peter Elsbach, NY University, New York, NY
MEMBRANE ACTIVE BACTERICIDAL PROTEIN FROM HUMAN AND RABBIT NEUTROPHILS

11:00 a.m.
David A. Bass, Bowman Gray School of Medicine, Winston-Salem, NC
MICROBICIDAL MECHANISMS OF EOSINOPHILS

11:30 a.m.
Paul G. Quie, University of Minnesota, Minneapolis, MN
CLINICAL SYNDROMES WITH IMPAIRED MICROBICIDAL FUNCTION

* Presentation by Pamela Shirley

TARGETED SESSION I

MACROPHAGES AND THE IMMUNE RESPONSE

Monday, October 18, 8:30-10:30 a.m.

Alan M. Kaplan presiding

STOCKHOLM ROOM

1. 8:30 a.m.

SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS) INHIBITS CYTOSKELETAL-DEPENDENT LYMPHOCYTE FUNCTION IN CULTURE AND MICROTUBULE ASSEMBLY IN VITRO. Richard D. Irons, R. W. Pfeifer*, T. M. Aune* and C. W. Pierce. Chemical Industry Institute of Toxicology, Dept. of Pathology, Research Triangle Park, NC 27709 and The Jewish Hospital of St. Louis, St. Louis, MO 63110.

2. 8:45 a.m.

SOLUBLE SUPPRESSOR FACTOR FOR ANTIBODY SYNTHESIS PRODUCED BY MITOGEN STIMULATED HUMAN MARROW CELLS. Charles D. Alley. Dept. of Anatomy, Univ. of Alabama, Birmingham, AL 35294.

3. 9:00 a.m.

ROLE OF ACCESSORY CELLS IN T COLONY FORMATION. Alan Winkelstein. Montefiore Hospital, Pittsburgh, PA 15213.

4. 9:15 a.m.

IA^+ P388AD TUMOR CELLS PRESENT ANTIGEN TO LONG-TERM ANTIGEN-SPECIFIC T CELL CULTURES. Donald A. Cohen* and A. M. Kaplan. Medical Microbiology and Immunology, Albert Chandler Med. Ctr., Univ. of Kentucky, Lexington, KY 40536.

5. 9:30 a.m.

INABILITY OF UREMIC MACROPHAGES TO PRESENT ANTIGEN TO CONTROL IMMUNE T CELLS. Yael G. Alevy*, Kathleen R. Mueller* and Raymond G. Slavin. Allergy and Immunology/Internal Medicine, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

6. 9:45 a.m.

IMMUNOLOGICAL STUDIES IN YOUNG HOMOSEXUAL MALES. Peter W. A. Mansell, J. M. Reuben*, G. R. Newell* and E. M. Hersh. Dept. of Cancer Prevention and Dept. of Clinical Immunology and Biological Therapy, M. D. Anderson Hospital, Houston, TX 77030.

7. 10:00 a.m.

ENHANCED PRODUCTION OF MURINE INTERFERON$_\gamma$ BY T-CELLS GENERATED IN RESPONSE TO BACTERIAL INFECTION. George L. Spitalny and Edward A. Havell*. Trudeau Institute, Inc., Saranac Lake, NY 12983.

TARGETED SESSION II

REGULATION OF INFLAMMATION

Monday, October 18, 8:30-10:30 a.m.

Robert North presiding

EMPIRE ROOM

8. 8:30 a.m.

HUMAN OVIDUCTAL(oM) AND PERITONEAL MACROPHAGES(pM) AS CELLULAR MEDIATORS OF INFERTILITY. A. F. Haney* and J. B. Weinberg. Veterans Administration Hospital and Duke University Med. Ctr. Durham, NC 27705.

9. 8:45 a.m.

INHIBITION OF HUMAN NEUTROPHIL ELASTASE BY HUMAN ALVEOLAR MACROPHAGE LYSATE: PARTIAL CHARACTERIZATION OF THE INHIBITORY ACTIVITY. M. S. Wald* and E. J. Campbell. Pulmonary Div., Dept. Medicine, Jewish Hospital St. Louis, St. Louis, MO 63110.

10. 9:00 a.m.

ROLE OF MACROPHAGES IN LOCAL IMMUNOREGULATION IN PREGNANT MICE. Gary W. Wood, L. Manning* and J. Hunt. Dept. Pathology and Oncology, Univ. Kansas Med. Ctr., Kansas City, KS 66103.

11. 9:15 a.m.

THE EFFECT OF PLASMA FIBRONECTIN-MEDIATED PHAGOCYTOSIS ON SUPEROXIDE PRODUCTION BY INFLAMMATORY NEUTROPHILS AND MACROPHAGES. Paul W. Gudewicz, C. M. Buys*, R. Kumar* and J. Molnar. Dept. Physiology, Albany Medical College, Albany, NY 12208 and Univ. Illinois at the Med. Ctr., Chicago, IL 60680.

12. 9:30 a.m.

FIBRONECTIN MEDIATED PHAGOCYTOSIS OF DENATURED COLLAGEN (GELATIN) COATED PARTICLES BY ISOLATED RAT KUPFFER CELLS. J. M. Cardarelli*, F. A. Blumenstock, F. J. Rourke* and T. M. Saba. Dept. Physiology, Albany Medical College, Albany, NY 12208.

13. 9:45 a.m.

TUFTSIN RESTORES THE DEPRESSED RETICULOENDOTHELIAL (RE) FUNCTION AFTER SPLENECTOMY (SPLY) AND IMPROVES SURVIVAL FOLLOWING SPLY AND SEPSIS. Irshad H. Chaudry. Dept. Surgery, Yale Univ. Sch. Medicine, New Haven, CT 06510.

14. 10:00 a.m.

PANCREATIC HORMONAL INFLUENCE OF KUPFFER CELL PHAGOCYTOSIS IN VIVO AND IN THE ISOLATED PERFUSED LIVERS OF RATS. Robert P. Cornell. Division of Science, Northeast Missouri State Univ., Kirksville, MO 63501.

TARGETED SESSION III

PULMONARY DEFENSE MECHANISMS

Monday, October 18, 8:30-10:30 a.m.

Carlos Daughaday presiding

GEORGIAN ROOM

15. 8:30 a.m.

IN VITRO ASSAY FOR MACROPHAGE MOVEMENT. M. A. Leyko*.(Introduced by: Michel Rabinovitch). Institute of Environmental Medicine, New York Univ. Med. Ctr., New York, NY 10016.

16. 8:45 a.m.

MACROPHAGE-DERIVED CHEMOTAXINS: ROLE IN THE LUNG'S INFLAMMATORY RESPONSE TO INTRATRACHEAL IRON OXIDE. James H. Dauber, E. Lugano* and R. Daniele. Pulmonary Service, Veterans Administration Medical Center, Pittsburgh, PA 15240 and Univ. Penn., Philadelphia, PA 19104.

17. 9:00 a.m.

DEACTIVATION OF ALVEOLAR MACROPHAGE RESPONSE TO CHEMOTACTIC FACTORS. Carlos C. Daughaday, J. Mehta*, A. N. Bohrer* and I. Spilberg*. Respiratory Care Center, Veterans Administration Medical Center and Washington Univ., St. Louis, MO 63125.

18. 9:15 a.m.

THE FATE OF PASTEURELLA HEMOLYTICA IN NORMAL MOUSE PERITONEAL AND ALVEOLAR MACROPHAGES. Frank M. Collins, S.S.Mtero*, C. J. Niederbuhl* and S. G. Campbell. Trudeau Institute, Inc., Saranac Lake, NY 12983 and Cornell Univ. Veterinary College, Ithaca, NY 14853.

19. 9:30 a.m.

CELLULAR AND SERUM ALTERATIONS INDUCED BY CHRONIC PSEUDOMONAS PULMONARY INFECTION. Glenna B. Winnie*, J. Klinger*, P. Cheng* and M. J. Thomassen. Pediatric Pulmonary Dept., Rainbow Babies & Childrens Hospital and Case Western Reserve Univ., Cleveland, OH 44106.

20. 9:45 a.m.

FREQUENCY OF BLACK PIGMENT IN LIVER AND SPLEEN OF COAL WORKERS. Marian E. Lefevre, F. H. Y. Green*, D. D. Joel and W. Laqueur*. Medical Dept., Brookhaven National Laboratories, Upton, NY 11973 and Pathology Section, NIOSH, Morgantown, WV 26505.

POSTER SESSION I

Monday, October 18, 10:30 a.m.- 12:15 p.m.

KHORASSAN A

21.

RAT MACROPHAGES REQUIRE Ia ANTIGEN TO RELEASE LEUKOCYTE ENDOGENOUS MEDIATOR (INTERLEUKIN-1). Roger H. Mitchell, N. J. Loving* and R. F. Kampschmidt. The Noble Foundation, Ardmore, OK 73401.

22.

POTENTIATION OF ALLOGRAFT RESPONSIVENESS BY PLASMA FIBRONECTIN. David B. Lause, J. E. Doran, J. A. Houston* and D. H. Beezhold. Dept. Anatomy, Medical College of Georgia, Augusta, GA 30912 and The Veterans Administration Medical Research Services, Augusta, GA 30910.

23.

HUMAN MONOCYTE MIGRATION INHIBITION (MMI) BY HUMAN INTERFERON (IFN) AND INTERFERON-INDUCERS. Gary B. Thurman and Henry C. Stevenson*. Biological Response Modifiers Program, Frederick Cancer Research Facility, Frederick, MD 21701.

24.

THE STUDY OF A MONOKINE THAT ENHANCES LYMPHOCYTE MIGRATION IN VITRO. H. Tak Cheung and J.S. Twu*. Dept. Biological Sciences, Illinois State Univ., Normal, IL 61761.

25.

INHIBITION OF IN VITRO LYMPHOCYTE RESPONSES BY VERY LOW DENSITY LIPOPROTEINS FROM DIABETIC RAT SERUM. David S. Chi, D. L. Berry*, K. A. Dillon* and B. W. Arbogast*. Quillen-Dishner College of Medicine, East Tennessee State Univ., Johnson City, TN 37614.

26.

CHARACTERIZATION OF ALANINE AND TYROSINE AMINOPEPTIDASE ACTIVITIES IN LUNG MACROPHAGES. Glenn A. Warr and P. L. Sannes*. Dept. Environmental Health Sciences, Johns Hopkins Univ. Sch. Hygiene and Public Health, Baltimore, MD 21205.

27.

PARAMETERS EFFECTING HEPATIC CLEARANCE AND KILLING OF MICROBES. Richard T. Sawyer, E. J. Cabrera*, K. J. Neumann* and M. Raveli*. Mercer Univ. School of Medicine, Macon, GA 31207, Norwich Pharmaceuticals, Norwich, NY and East Carolina Univ. School of Medicine, Greenville, NC 27834.

28.

NON-SPECIFIC MITOGENIC RESPONSES OF LEW AND F344 RAT LYMPHOCYTES TOWARD MYCOPLASMA PULMONIS. J. K. Davis*, J. W. Simecka*, R. B. Thorp* and G. H. Cassell. Dept. Microbiology, Univ. Alabama, Birmingham, AL 35294.

29.

LYSOSOMAL ENZYMES (LE) AND MICROBICIDAL CAPACITIES OF ALVEOLAR MACROPHAGES IN PULMONARY DEFENSE MECHANISMS. Saroj Chandrasekhar and M. K. Mukerji*. Dept. Microbiology, V.P. Chest Institute, Univ. Delhi, Delhi, India 110007.

TARGETED SESSION IV

CYTOTOXICITY: MACROPHAGE AND NATURAL KILLER CELLS

Tuesday, October 19, 8:30-10:30 a.m.

Howard Holden presiding

STOCKHOLM ROOM

30. 8:30 a.m.

PROGRESS IN THE PURIFICATION AND CHARACTERIZATION OF MACROPHAGE CYTOTOXIN (MCT). Jim Klostergaard, T. H. Reidarson* and G. A. Granger*. Dept. Molecular Biology and Biochemistry, Univ. California, Irvine, CA 92717.

31. 8:45 a.m.

SIMULTANEOUS MEASUREMENTS OF CYTOSTASIS AND CYTOTOXICITY USING THE FLOW CYTOMETER. A. P. Stevenson, J. C. Martin* and C. C. Stewart. Experimental Pathology, Los Alamos National Laboratories, Los Alamos, NM 87545.

32. 9:00 a.m.

DIFFERENTIAL CYTOTOXIC EFFECTS OF PERITONEAL MACROPHAGES AND THE J774 MONOCYTIC CELL LINE ON METASTATIC VARIANTS OF MURINE B16 MELANOMA AND RAW 117 LYMPHOSARCOMA IN VITRO. Karen M. Miner*, J. Klostergaard, G. A. Granger* and G. L. Nicolson* (Introduced by: P. Davies). Merck Institute, Rahway, NJ 07065, Dept. Molecular Biology and Biochemistry, Univ. California, Irvine, CA 92717 and Dept. Tumor Biology, M.D. Anderson Hospital, Houston, TX 77030.

33. 9:15 a.m.

DISTINCT REGULATORY MECHANISMS FOR ANTIBODY-DEPENDENT (ADCC) AND NONSPECIFIC KILLING OF TUMOR TARGETS BY MURINE AND HUMAN MACROPHAGES, AND ROLE OF IG CLASS IN ADCC. Peter Ralph, I. Nakoinz*, N. Williams*, C. Punjabi* and P. Litcofsky*. Sloan-Kettering Institute, Rye, NY 10580.

34. 9:30 a.m.

THE RELATIONSHIP BETWEEN NATURAL KILLER CELLS AND EFFECTOR CELLS INVOLVED IN LECTIN DEPENDENT CELLULAR CYTOTOXICITY AND ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY IN MAN. Thomas P. Bradley* and B. Bonavida. Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024.

84. 9:45 a.m.

ASSOCIATION OF CYTOLYTIC ACTIVATION OF MURINE MACROPHAGES WITH DECREASED LEVELS OF 28S RIBOSOMAL RNA. Luigi Varesio and R. Kowall*. National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701.

35. 10:00 a.m.

ALTERED SENSITIVITY TO MACROPHAGE-MEDIATED CYTOTOXICITY IN PRIMARY AND METASTASIS-DERIVED MURINE TUMOR CELL LINES. Fred P. Nestel*, R. H. Wiltrout* and R. S. Kerbel*(Introduced by: H. F. Pross). Cancer Research Labs, Queen's Univ., Kingston, Ontario, Canada K7L 3N6.

36. 10:15 a.m.

TIME-RESPONSE CHARACTERISTICS OF THE CELLULAR IMMUNE RESPONSE TO THE H-1 ANTIGEN. M. Kurtz*, D. Martin*, H. Russell* and R. J. Graff*(Introduced by: David Thomasson). Research Dept., John Cochran Veterans Administration Hospital, St. Louis, MO 63106 and Washington Univ.Sch. of Med., St. Louis, MO 63110.

TARGETED SESSION V

REGULATION OF MACROPHAGE FUNCTION AND SECRETION

Tuesday, October 19, 8:30-10:30 a.m.

Joseph W. Shands presiding

EMPIRE ROOM

37. 8:30 a.m.

DIFFERENTIAL ADJUVANT ACTIVATION OF MACROPHAGES FROM YOUNG AND AGING MICE. Pamela Petrequin and A. G. Johnson. Dept. Microbiology, School of Medicine Univ. Minnesota, Duluth, MN 55812.

38. 8:45 a.m.

ACTIVATION OF ADENYLATE CYCLASE (AC) IN GUINEA PIG MACROPHAGE MEMBRANES IS REGULATED BY GUANINE NUCLEOTIDES. Margrith Verghese* and Ralph Snyderman. Duke Univ. Medical Center, Durhan, NC 27710.

39. 9:00 a.m.

MACROPHAGE RECEPTORS FOR PHORBOL DIESTERS: COMPARABLE RECEPTORS IN NORMAL AND "ACTIVATED" CELLS DESPITE DIFFERENT CELLULAR RESPONSES. J. Brice Weinberg. Veterans Administration Hospital, Durhan, NC 27705.

40. 9:15 a.m.

MOUSE MACROPHAGE PROCOAGULANT(S): EVIDENCE FOR VITAMIN K DEPENDENCE. Joseph W. Shands. Div. Infectious Diseases, Univ. Florida, Gainesville, FL 32610.

41. 9:30 a.m.

CHARACTERIZATION OF THE PROCOAGULANT ACTIVITY (PCA) INDUCED IN RABBIT KUPFFER CELLS(KC) BY BACTERIAL LIPOPOLYSACCHARIDE(LPS). Ronald V. Maier*(Introduced by: E. Stan Lennard). Harborview Medical Center, Seattle, WA 98104.

42. 9:45 a.m.

INHIBITION OF MURINE MACROPHAGE MIGRATION BY THE LEUKOCYTOSIS PROMOTING TOXIN (LPT) OF BORDETELLA PERTUSSIS. Bruce D. Meade, P. D. Kind* and C. R. Manclark*(Introduced by: S. K. Ackerman). Pertussis Branch, DBP, Bureau of Biologics, Bethesda, MD 20205 and George Washington Univ., Washington, DC 20037.

TARGETED SESSION VI

ANTIMICROBIAL FUNCTIONS

Tuesday, October 19, 8:30-10:30 a.m.

Michael Rabinovitch presiding

GEORGIAN ROOM

8:30 a.m. INTRODUCTION. Michael Rabinovitch, New York Univ. Medical Center, NY, NY.

43. 8:45 a.m.

NEUTRALIZATION OF HOST DEFENSE MECHANISMS BY GBS CAROTENOID PIGMENT. Ruth A. Nemergut* and K. Merritt. Orthopaedic Research, Univ. California, Davis, CA 95616.

44. 9:00 a.m.

RADIOIODINATED β-GLUCURONIDASE ENTERS THE PARASITOPHOROUS VACUOLES OF MACROPHAGES INFECTED WITH LEISHMANIA MEX. AMAZONESIS. Virginia L. Shepherd*, P. D. Stahl, D. Biegel*, G. Topper* and M. Rabinovitch. Dept. Physiology and Biophysics, Washington Univ. Medical School, St. Louis, MO 63110 and NYU Sch. Med., Dept. Cell Biology, New York, NY 10016.

45. 9:15 a.m.

OXYGEN-INDEPENDENT ACTIVITY AGAINST INTRACELLULAR PATHOGENS: TOXOPLASMA GONDII AND CHLAMYDIA PSITTACI. Gerald I. Byrne*, C. D. Rothermel* and H. W. Murray.Dept. Medicine, Div. International Medicine, Cornell Univ. Med. College, New York, NY 10021.

46. 9:30 a.m.

KILLING OF LISTERIA MONOCYTOGENES BY INFLAMMATORY NEUTROPHILS AND MONONUCLEAR PHAGOCYTES OBTAINED FROM IMMUNE AND NON-IMMUNE MICE. Charles J. Czuprynski, P. Henson and P.A. Campbell. Dept. Medicine, National Jewish Hospital and Research Center, Denver, CO 80206.

47. 9:45 a.m.

THE IDENTIFICATION OF LYMPHOKINES PRODUCED BY A HUMAN T CELL LINE WHICH INHIBIT OR ENHANCE THE INTRAMACROPHAGE REPLICATION OF MYCOBACTERIUM TUBERCULOSIS, OR INDUCE THE FORMATION OF MULTINUCLEATE GIANT CELLS, IN HUMAN MACROPHAGE CULTURES. G. S. Douvas*, D. Anderson* and A. J. Crowle. Webb-Waring Lung Institute, Univ, Colorado Health Science Center, Denver, CO 80262.

48. 10:00 a.m.

MODULATION OF THE EEL IMMUNE SYSTEM BY ETE. L. J. McCumber*, M. M. Sigel, E. Huggins*, J. Davis* and S. Hayasaka*. Microbiology and Immunology, School of Medicine Univ. South Carolina, Columbia, SC 29208 and Dept. Microbiology, Clemson Univ, Clemson, SC 29631.

POSTER SESSION II

Tuesday, October 19, 11:00 a.m.- 12:30 p.m.

KHORASSAN A

49.

REACTIVITY OF ANTIBODY TO ASIALO-GM1 WITH ELICITED AND ACTIVATED MACROPHAGES. R. H.Wiltrout, A. Santoni, E. S. Peterson, R. B. Herberman and H. T. Holden. Biological Research and Therapy Branch, NCI-FCRF, Frederick, MD 21701.

50.

3':5' CYCLIC ADENOSINE MONOPHOSPHATE REGULATES HAMSTER NATURAL KILLERS AT TWO DIFFERENT LEVELS. Samuel Fan* and Wayne Tompkins. Dept. Veterinary Pathobiology, Univ. Illinois, Urbana, IL 61801.

51.

DISSOCIATION OF NK ACTIVITY AND RESISTANCE TO URETHANE-INDUCED LUNG ADENOMAS. Suzanne Lemieux* and Emil Skamene. Institut Armand-Frappier, Univ. Quebec, Laval-Des-Rapides, Quebec, Canada H7V 1B7 and Montreal General Hospital, Montreal, Quebec, Canada, H36 1A4.

52.

ROLE OF NK CELLS IN THE INHIBITION OF TUMOR GROWTH BY INTERFERON. Timothy L. Ratliff, Dov Kadmon*, Dennis M. Oakley*, Amos Shapiro* and William J. Catalona*. Dept. Surgery(Urology), The Jewish Hospital of St. Louis, St. Louis, MO 63110.

53.

EFFECTS OF FASTING ON IMMUNITY IN HUMANS: DISSOCIATION OF CELLULAR FUNCTION. Edward J.Wing, A.W. Winkelstein. Montefiore Hospital, Pittsburgh, PA 15213.

54.

EFFECT OF CA^{++} ION INFLUX AND CALMODULIN ACTIVITY ON MACROPHAGE CYTOTOXICITY. Hue Duy*, W. Tompkins. Dept. Veterinary Pathobiology, Univ. Illinois, Urbana, IL 61801.

55.

REQUIREMENT OF GLYCOSYLATED PROTEIN FOR HUMAN NK EFFECTOR CELL RECOGNITION. Ann Hinson*, Edwin W. Ades. Lilly Research Labs, Eli Lilly and Company, Indianapolis, IN 46285.

56.

IMMUNOLOGIC AND VIRAL PROTECTIVE PROPERTIES OF BORDETELLA PERTUSSIS ACELLULAR FRACTIONS 15A-1B AND 15A-108A (CONNAUGHT LABORATORIES, SWIFTWATER, PA). Robert S. Stinson, J. Dennis Lee*, Lisa Williamson* and Alvin Winters*. Dept. Microbiology, Univ. Alabama, University, AL 35486.

57.

THE INDUCTION OF COMPLEMENT RECEPTOR 3 (CR3) ON MACROPHAGES BY AN IN VIVO-DERIVED DIFFERENTIATION ACTIVITY. Shing-Erh Yen* and W. S. Walker. Dept. Immunology, St. Jude Childrens Research Hospital, Memphis, TN 38101.

85.

SPONTANEOUS CYTOTOXICITY BY HUMAN ADHERENT PERIPHERAL BLOOD MONONUCLEAR CELLS AGAINST HUMAN OR MOUSE ANCHORAGE-DEPENDENT TUMOR CELL LINES: CONTRIBUTIONS OF ADHERENT NK-LIKE CELLS. Z-L Chang*, R. Hoffman*, E. Bonvini*, H. C. Stevenson* and R. B. Herberman. Biological Development, NCI-FCRF, Frederick, MD 21701.

87.

IMMUNOSUPPRESSIVE FACTORS IN EHRLICH ASCITES CARCINOMA FLUID INHIBIT THE MITOGENIC RESPONSE OF NORMAL LYMPHOID CELLS. J. Quddus* and R. E. McCarthy. Dept. Medical Microbiology, Univ. Nebraska Med. Ctr., Omaha, NE 68105.

89.

GUINEA PIG MONOCYTE SUBPOPULATIONS DEMONSTRATE DIFFERENCES IN NATIVE TUMORICIDAL ACTIVITY. S.J. Noga* and S.J. Normann. Dept. Pathology, University Florida, Gainesville, FL 32610.

TARGETED SESSION VII

HOST RESPONSE TO TUMORS

Tuesday, October 19, 1:30-3:30 p.m.

Stephen W. Russell presiding

STOCKHOLM ROOM

1:30 p.m. INTRODUCTION. Stephen W. Russell, Univ. Florida, Gainesville, FL.

58. 1:45 p.m.

T CELL HYBRIDOMA PRODUCTION OF LYMPHOKINE ACTIVITY THAT INTERFERES WITH PGE_2-MEDIATED NEGATIVE REGULATION OF MACROPHAGE ACTIVATION. Stephen W. Russell and J. L. Pace*. Comparative Experimental Pathology, Univ. Florida, Gainesville, FL 32610.

59. 2:00 p.m.

THE ROLE OF MACROPHAGES AND FUNCTIONAL T-CELL SUBSETS IN THE LEUKOCYTE(MACROPHAGE) ADHERANCE INHIBITION(LAI) MEASURE OF CELLULAR IMMUNITY TO MURINE SARCOMA VIRUS(MSV)-INDUCED TUMORS. Katherine Sarlo* and Richard F. Mortensen. Dept. Microbiology, Ohio State Univ., Columbus, OH 43210.

60. 2:15 p.m.

PURIFICATION OF MULTIPLE HUMAN MACROPHAGE ACTIVATION FACTORS (MAF). Mildred C. McDaniel. Quillen-Dishner College of Medicine, East Tennessee State Univ., Johnson City, TN 37614.

61. 2:30 p.m.

INFILTRATION OF HOST DEFENSE CELLS IN TUMORS. Carleton C. Stewart, S. J. Stewart* and A. P. Stevenson. Experimental Pathology, Los Alamos National Laboratories, Los Alamos, NM 87545.

62. 2:45 p.m.

FIBRONECTIN LEVELS DURING GROWTH OF THE LEWIS LUNG TUMOR AS RELATED TO MACROPHAGE FUNCTION. Robert Megirian, F. A. Blumenstock, J. A. Bennett* and T. M. Saba. Dept. Physiology and Surgery, Albany Medical College, Albany, NY 12208.

63. 3:00 p.m.

THE EFFECT OF DIETHYLSTILBESTROL(DES) ON HOST RESISTANCE AND TUMOR SUSCEPTIBILITY IN MICE. Jeannie N. Bradof*, R. Fugmann, C. Aranyi*, P. Barbera* and J. Fenters*. ITT Research Institute, Life Sciences Division, Chicago, IL 60616.

64. 3:15 p.m.

MODIFICATION OF A LYMPHOID CELL SUBSET IN PATIENTS WITH BREAST CANCER. Mariano F. Lavia, E. Cillari*, G. Di Gesu*, S. Palmeri*, D. Lio*, A. Salerno* and P. LiVoti*. Medical Univ. of South Carolina, Charleston, SC 29425 and Univ. Palermo, Italy.

TARGETED SESSION VIII

MACROPHAGE DIFFERENTIATION AND PROLIFERATION

Tuesday, October 19, 1:30-3:45 p.m.

Robert Musson presiding

EMPIRE ROOM

65. 1:30 p.m.

ANTIGEN-INDUCED PROLIFERATION OF THIOGLYCOLATE-ELICITED MOUSE PERITONEAL MACROPHAGES IN VITRO UNDER NORMAL CULTURAL CONDITIONS. Paul L. Glover* and Eugene H. Perkins. Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830.

66. 1:45 p.m.

LONG TERM HUMAN PERIPHERAL BLOOD MONOCYTE CULTURES-EXPRESSION OF HLA,Ia AND MONOCYTE ANTIGENS. Steven D. Douglas, N. E. Kay, M. E. Moore* and S. Ferrone*. Div. Allergy, Immunology

and Pulmonology, The Childrens Hospital Philadelphia, Philadelphia, PA 19104, Veterans Administration Med. Ctr., Minneapolis, MN 53417 and Columbia Univ. College of Physicians and Surgeons, New York, NY 10032.

67. 2:00 p.m.

SERUM-DEPENDENT MATURATION OF HUMAN MONOCYTES IN VITRO. Robert A. Musson. National Jewish Hospital and Research Center, National Asthma Center, Denver, CO 80206.

68. 2:15 p.m.

HISTOCHEMICAL AND FUNCTIONAL ANALYSES OF MULTINUCLEATED GIANT CELLS DERIVED IN VITRO FROM HUMAN MONOCYTES. L. Schlesinger*, R. A. Musson and R. B. Johnston, Jr., National Jewish Hospital and Research Center, National Asthma Center, Denver, CO 80206.

69. 2:30 p.m.

CHEMOTACTIC RESPONSES OF HUMAN MONOCYTES THAT REPOPULATE THE CIRCULATION DURING MONOCYTE DEPLETION BY LEUKOPHERESIS. Enrica Alter* and Edward J. Leonard. Laboratory of Immunobiology, National Cancer Institute, Bethesda, MD 20205.

70. 2:45 p.m.

IDENTIFICATION, ENRICHMENT AND PARTIAL CHARACTERIZATION OF FOLLICULAR DENDRITIC CELLS FROM MOUSE LYMPH NODES IN VITRO. Carol T. Schnizlein*, K. L. Holmes*, N. L. Keesling* and J. G. Tew. Microbiology and Immunology, Medical College of Virginia-VCU, Richmond, VA 23298.

71. 3:00 p.m.

AN INVESTIGATION OF THE "NEURAL SPECIFIC" S-100 PROTEIN AS A CYTOPLASMIC MARKER FOR DENDRITIC CELLS. Geoffrey Rowden, Y. Yamamura*, T. Misra*, K. Sheikh*, E. Connelly and H. Higley*. Dept. Pathology, Loyola Medical Center, Maywood, IL 60153.

86. 3:15 p.m.

PHENOTYPE OF THE HAIRY CELLS OF LEUKEMIC RETICULOENDOTHELIOSIS DEFINED BY MONOCLONAL ANTIBODIES. Anthony J. Janckila, G. T. Stelzer, J. H. Wallace* and L. T. Yan*. Veterans Administration Medical Center, Dept. Microbiology and Immunology, Univ. Louisville and The Histocompatability Laboratory, Jewish Hospital, Louisville, KY 40202.

3:30 SUMMATION. Robert Musson, National Jewish Hospital, Denver, CO 80206

TARGETED SESSION IX

MACROPHAGE HETEROGENEITY

Tuesday, October 19, 3:45-5:30 p.m.

William S. Walker presiding

STOCKHOLM ROOM

3:45 p.m. INTRODUCTION. William S. Walker, St. Jude Children's Research Hosp., Memphis, TN 38101.

72. 4:00 p.m.

MOUSE MONONUCLEAR PHAGOCYTE ANTIGENS DETECTED BY MONOCLONAL ANTIBODIES USING IMMUNO-FLUORESCENCE AND COMPLEMENT DEPENDENT CYTOTOXICITY. Paul A. Leblanc*, Shou-Mei T. Chang* and Stephen W. Russell. Comparative Pathology, Univ. Florida, Gainesville, FL 32610.

73. 4:15 p.m.

BONE MARROW ORIGIN OF MACROPHAGE HETEROGENEITY. Isia Bursuker* and R. Goldman. Trudeau Institute, Inc., Saranac Lake, NY 12983 and Dept. Membrane Research, The Weizman Institute of Science, Rehovot, Israel.

74. 4:30 p.m.

HETEROGENEITY WITHIN RAT PERITONEAL MACROPHAGE SUBPOPULATIONS. R. H. J. Beelen*, E. C. M. Hoefsmit* and W. S. Walker. Dept. Electron Microscopy, Faculty Medicine, Free Univ., Netherlands and Div. Immunology, St. Jude Children's Research Hospital, Memphis, TN 38101.

75. 4:45 p.m.

HETEROGENEITY AMONG PERITONEAL MACROPHAGES. Eugene P. Mayer and A. Ghaffar. Microbiology and Immunology, School of Medicine Univ. South Carolina, Columbia, SC 29208.

76. 5:00 p.m.

DISSOCIATION OF TUMORICIDAL AND BACTERICIDAL ACTIVITIES WITHIN MOUSE PERITONEAL CELL POPULATIONS. Priscilla A. Campbell, Charles J. Czuprynski and James L. Cook*. Dept. Medicine, National Jewish Hospital and Research Center, Denver, CO 80206.

77. 5:15 p.m.

PHYSICAL SEPARATION OF RABBIT ALVEOLAR MACROPHAGES INTO FUNCTIONAL SUBPOPULATIONS. M. A. Murphy* and H. B. Herscowitz. Dept. Microbiology, Georgetown Univ. Med. Ctr., Washington, DC 20007.

TARGETED SESSION X

NEUTROPHIL FUNCTION

Tuesday, October 19, 3:45-5:30 p.m.

Samuel K. Ackerman presiding

EMPIRE ROOM

78. 3:45 p.m.

EFFECTS OF 2-DEOXY-D-GLUCOSE (2DOG) ON CHEMOTACTIC MODULATION OF NEUTROPHIL ADHESIVENESS. C. Wayne Smith, J. C. Hollers* and D. C. Anderson. Dept. Anatomy, Michigan State Univ., East Lansing, MI 48824 and Dept. Pediatrics, Baylor College of Medicine, Houston, TX 77030.

79. 4:00 p.m.

FLOW CYTOMETRIC STUDIES OF OXIDATIVE PRODUCT FORMATION BY NEUTROPHILS: A GRADED RESPONSE TO MEMBRANCE STIMULATION. David A. Bass, J. W. Parce*, P. Szejda*, M. C. Seeds*, M. Thomas* and L. R. DeChatelet. Bowman Gray School of Medicine, Winston-Salem, NC 27103.

80. 4:15 p.m.

RECEPTOR BLOCKADE AS A MECHANISM OF CROSS-DEACTIVATION OF HUMAN NEUTROPHILS. Jagdish Mehta*, M. Muniain*, I. Spilberg*, L. Simchowitz* and J. Atkinson*(Introduced by: C.C. Daughaday). Rheumatology Unit, Veterans Administration Medical Center and Washington Univ. Med. School, St. Louis, MO 63106.

81. 4:30 p.m.

EFFECTS OF TAXOL ON POLYMORPHONUCLEAR LEUKOCYTE FUNCTION. Ronald S. Oseas* and E. Toloza* (Introduced by: Michael E. Miller). Harbor-UCLA Medical Center, Torrance, CA 90509.

82. 4:45 p.m.

INHIBITION OF NEUTROPHIL PHAGOCYTOSIS BY BACTERIAL CULTURE FILTERATES. D. G. Paarlberg*, L. A. Di Pietro*, R. G. Crispen and T. Y. Sabet. Dept. Histology, Univ. Illinois College of Dentistry and Institute of Tuberculosis Research, Chicago, IL 60612.

83. 5:00 p.m.

EFFECTS OF A MODIFIED LIVE BVD VIRUS VACCINE AND ACTH ON BOVINE GRANULOCYTE FUNCTION. James A. Roth and M. L. Kaeberle*. Dept. Veterinary Microbiology and Preventative Medicine, Iowa State Univ., Ames, IA 50011.

88. 5:15 p.m.

IMPAIRED CHEMOTAXIS RELATED TO ABNORMALITIES OF PMN LEUKOCYTE ADHERENCE IN PROTEIN CALORIE MALNUTRITION (PCM). D. C. Anderson, G. S. Krishna*, B. Hughes*, M. L. Mace*, B. L. Nichols* and C. W. Smith. Childrens Nutrition and Research Ctr., Baylor College Med., Houston, TX 77030 and Dept. of Anatomy, Michigan State Univ., Lansing, MI 48864.

Scientific Program: 19th National Meeting Reticuloendothelial Society*

Chase Park Plaza Hotel
St. Louis, Missouri
October 17–20, 1982

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Timothy L. Ratliff, Jewish Hospital, St. Louis
Phil D. Stahl, Washington University School of Medicine

*A Pre-meeting laboratory workshop on *Macrophage and Lymphocyte Functions* will be held on October 16 and 17 at the Washington University Student Laboratories. Dr. Herbert B. Herscowitz, Georgetown University, Dr. Philip D. Stahl, Washington University, Organizers.

Program

OCTOBER, 17

2:00 p.m.–5:00 p.m. *Discussion Workshop—Nomenclature of Natural Killer and Natural Cytotoxic Cells* — Chase Club

Dr. Ronald B. Herberman, NCI, NIH, Chair

Participation in this workshop is open to all individuals interested in natural cell mediated cytotoxicity. Individuals who have worked with NK cells, particularly heterogeneity of effector cells, are invited to bring 1 or 2 slides to illustrate their work.

7:00 p.m.–8:00 p.m. OPENING PROGRAM — Chase Club

Keynote Address: Dr. Ira Pastan

Chief, Laboratory of Molecular Biology, NCI, NIH

*"Role of the Receptosome in Receptor-Mediated Endocytosis"**

*Studies in collaboration with Dr. Mark Willingham

8:00 p.m.–10:30 p.m. *Symposium I—Mononuclear Phagocyte Plasma Membranes and Receptors* — Chase Club

Dr. Paul J. Edelson, Harvard University Medical School, Chair

1. Ectoenzymes—Dr. Paul J. Edelson
2. Modulation of the Mannose Specific Endocytosis of Macrophages—Dr. Philip Stahl, Washington University
3. Receptors for Chemotactic Peptides—Dr. E. Schiffman, NIH

10:00 p.m.–11:30 p.m. MIXER — Chase Lounge

OCTOBER 18

8:30 a.m.–10:30 a.m. Targeted Sessions

1. Macrophages and the Immune Response, Papers 1–7
   A. Kaplan, Chair — Stockholm
2. Regulation of Inflammation, Papers 8–14
   R. North, Chair — Empire
3. Pulmonary Defense Mechanisms, Papers 15–20
   C. Daughaday, Chair — Georgian

11:00 a.m.–12:30 p.m. Poster Session I, Papers 21–29 — Khorassan A

Exhibits — Khorassan A

12:30 p.m.–2:00 p.m. Selected Topics Luncheon — Mr. Sam's

2:00 p.m.–5:00 p.m. *Symposium II—Lipid Related Secretory Functions of Mononuclear Phagocyte* — Chase Club

Dr. Philip Davies, Merck Institute for Therapeutic Research, Rahway, NJ, Chair

1. Metabolism of Lipoproteins by Monocyte-Macrophages – Dr. Daniel Steinberg, University of California, San Diego
2. Regulation of Prostaglandin and Leukotriene Production by Macrophages – Dr. John Humes, Merck Institute for Therapeutic Research, Rahway, NJ
3. Release of PAF and other Lipid Derived Mediators – Dr. Peter Henson, National Jewish Hospital, Denver
4. Fc Receptor Mediated Regulation of Macrophage Function – Dr. Tsueno Suzuki, University of Kansas Medical Center, Kansas City

5:15 p.m. Beer and Barbecue - Anheuser-Busch — Forest Park

OCTOBER 19

8:30 a.m.–10:30 a.m. Targeted Sessions

4. Cytotoxicity: Macrophage and Natural Killer Cells, Papers 30–36, 84
   H. Holden, Chair — Stockholm
5. Regulation of Macrophage Function and Secretion, Papers 37–42
   J. Shands, Chair — Empire
6. Antimicrobial Function, Papers 43–48
   M. Rabinovitch, Chair — Georgian

11:00 a.m.–12:30 p.m. Poster Session II, Papers 49–57, 85, 87 — Khorassan A

Exhibits — Khorassan A

1:30 p.m.–3:45 p.m. Targeted Sessions

7. Host Response to Tumors, Papers 58–64
   S. Russell, Chair — Stockholm
8. Macrophage Differentiation and Proliferation, Papers 65–71, 86
   R. Musson, Chair — Empire

3:45 p.m.–5:30 p.m. Targeted Sessions

9. Macrophage Heterogeneity, Papers 72–77
   W. Walker, Chair — Stockholm
10. Neutrophil Function, Papers 78–83, 88
    S. Ackerman, Chair — Empire

5:30 p.m.–6:30 p.m. Business Meeting — Empire

7:00 p.m.–9:00 p.m. Reception — Zodiac Room

Society Banquet; Awards — Starlight Room

OCTOBER 20

8:30 a.m.–12 noon *Lawrence R. DeChatelet Memorial Symposium – Phagocyte Microbicidal Mechanisms* — Chase Club

Dr. John K. Spitznagel, Emory University, Chair

1. Genetic Analysis as a Probe of Macrophage Microbicidal Responses – Dr. Emil Skamene, McGill University
2. Oxidative Bactericidal Mechanisms in Neutrophils – Dr. Lawrence R. DeChatelet, Bowman Gray School of Medicine
3. Non-Oxidative Bactericidal Mechanisms in Neutrophils – Dr. John K. Spitznagel, Emory University
4. Membrane Active Bactericidal Protein From Human and Rabbit Neutrophils – Dr. Peter Elsbach, New York University
5. Microbicidal Mechanisms of Eosinophils – Dr. David A. Bass, Bowman Gray School of Medicine
6. Clinical Syndromes With Impaired Microbicidal Function – Dr. Paul G. Quie, University of Minnnesota

Abstracts

1

SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS) INHIBITS CYTOSKELETAL-DEPENDENT LYMPHOCYTE FUNCTION IN CULTURE AND MICROTUBULE ASSEMBLY IN VITRO. R.D. IRONS, R.W. PFEIFER, T.M. AUNE AND C.W. PIERCE. Departments of Pathology, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709 and The Jewish Hospital of St. Louis, St. Louis, MO 63110.

Soluble immune response suppressor (SIRS) is a product of murine T cells that, when activated by macrophages or H_2O_2, suppresses a number of lymphocyte functions including: primary and secondary antibody responses, proliferative responses to mitogens and protein secretion. Because SIRS-induced suppression parallels that seen using known cytoskeletal disrupting agents, we examined its effects on cytoskeletal-dependent lymphocyte function in culture and microtubule (MT) assembly in vitro. Short term incubation of lymphocytes with SIRS, obtained from T cell hybridoma cultures and activated with H_2O_2, suppressed phytohemagglutinin-induced blastogenesis and partially protected against concanavalin A-inhibition of lymphocyte surface receptor redistribution (capping) at the same concentrations (50-100 µg/ml). In addition, column purified SIRS, activated with H_2O_2, inhibited purified porcine brain MT assembly in vitro in a concentration dependent manner (5-50 µg/ml). These effects of SIRS were protected against by dithiothreitol (10^{-4}-10^{-3}M), a sulfhydryl compound, in a manner similar to those of known sulfhydryl-reactive cytoskeletal disrupting agents (eg. N-ethylmaleimide, cytochalasin A, p-benzoquinone). These data suggest that the mechanism of SIRS-induced lymphocyte suppression may involve interference with sulfhydryl-dependent cytoskeletal events critical for cell function in vivo. This work was supported in part by NIH grant #AI15313 and NSF grant #PCM8119606.

2

SOLUBLE SUPPRESSOR FACTOR FOR ANTIBODY SYNTHESIS PRODUCED BY MITOGEN STIMULATED HUMAN MARROW CELLS. C.D. Alley. Department of Anatomy, University of Alabama in Birmingham, Birmingham, AL 35294.

We have previously reported the spontaneous synthesis and secretion of immunoglobulin by human bone marrow mononuclear cells (MNC) in vitro and its suppression by the addition of pokeweed mitogen (PWM). In the present study we demonstrate that this suppression is mediated by a soluble factor elaborated by marrow MNC stimulated with PWM. Marrow MNC were incubated for 1 hr with PWM, washed and recultured for 7d in media without PWM. The culture supernatants were collected by centrifugation and filter sterilized before addition to fresh marrow MNC in the antibody synthesis assay. After 14d, the culture supernatants were assayed for immunoglobulin by a solid phase radioimmunoassay. The suppressor substance was produced by nonadherent, non-T cells, was nonspecific as to immunoglobulin isotype and target cell origin. The addition of indomethacin had no effect on the suppression indicating that the activity was not mediated by prostaglandin. The suppressor substance was heat stable to 80°C, trypsin sensitive and could be elaborated by cells that were subjected to 1000R before or after 1 hr incubation with PWM. In addition to PWM, Concanavalin A and the erythro-agglutinating fraction of phytohemagglutinin were effective in producing suppressive activity in a population of marrow MNC. Lipopolysaccharide was not observed to induce suppressive activity. When MNC, before and after the pulse with PWM and subsequent 7d culture, were investigated for surface markers using monoclonal antibodies there was a significant increase in the proportion of the cells that were $DR.1^+$(40%). However, there were <3% $OKT3^+$, <1% $OKT4^+$, <1% $OKT8^+$ and <2% $Mac120^+$ cells throughout the period of incubation.

3

ROLE OF ACCESSORY CELLS IN T COLONY FORMATION. A. WINKELSTEIN. Department of Medicine, Montefiore Hospital, University of Pittsburgh, Pittsburgh, Pennsylvania 15213.

PHA stimulated human T lymphocytes, when plated in agar, undergo proliferative expansion, leading to the formation of discrete colonies. Using optimal growth conditions, the average colony number formed after plating 7.5 x 10^5 cells is 6,103 ±

539 (SEM) colonies/plate. Following separation of cells on Sephadex G-10 columns, the ability of non-adherent (NA) cells to form colonies is virtually abolished. Re-addition of irradiated adherent (ADH) cells results in a dose-dependent restoration of colony growth; maximum numbers of colonies were formed with a NA:ADH ratio of 2:1 (4,993 ± 497 colonies/plate). Direct contact was not essential; growth could be stimulated by plating the ADH cells in a hard agar underlayer. Conditioned media prepared from ADH also proved capable of promoting T colony formation. The functions of ADH could be supplied by several tumor cell lines including Raji cells (a B cell lymphoma) and U937 cells (a monocytoid line). Comparatively, Raji cells were more active than ADH; using a 1:1 ratio, the number of colonies averaged 10,865 ± 1,097. By contrast, K562 cells, an erythroleukemia line, were not active. Soluble factors prepared from both Raji and U937 cells were highly stimulatory. By contrast, T cell growth factor did not promote growth. These results suggest that the ability of PHA stimulated T cells to undergo clonal expansion depends upon soluble factors released from adherent cells. Similar growth promoting factors can be derived from both monocyte and B cell tumor lines.

4

Ia$^+$ P388AD TUMOR CELLS PRESENT ANTIGEN TO LONG-TERM ANTIGEN-SPECIFIC T CELL CULTURES D. A. COHEN* AND A. M. KAPLAN. University of Kentucky Medical College, Lexington, KY 40536-0084.

We have previously reported that an Ia$^+$ adherent tumor cell line, P388AD, but not the related Ia$^+$ nonadherent tumor cell, P388NA, presented the soluble protein antigen (Ag), turkey gamma globulin (TGG), to primed T cells. Although endogenous antigen-presenting cells (APC) were removed from the primed T cells with anti-Ia antiserum and complement, the possibility remained that Ia-negative macrophages (MØ) might participate in Ag presentation by P388AD. This report extends our previous studies by demonstrating that P388AD presents Ag to TGG-specific long-term T cell cultures which are devoid of contaminating MØ. Both the expression of surface Ia molecules and synthesis of interleukin-1 (IL-1) are required for Ag presentation by tumor cells in that only P388AD (Ia$^+$, IL-1$^+$) presents Ag, whereas P388NA (Ia$^+$, IL-1$^-$) and the well characterized MØ tumor P388D1 (Ia$^-$, IL-1$^+$) do not present Ag. However, when purified IL-1 is added to the cultures, P388NA behaves as an APC. Evidence that Ia is directly involved in the Ag presentation process comes from the observation that alloantisera directed against the specificities I-A.11,16 but not serum against the unrelated specificity IA.2 block presentation in a dose related manner. Ag presentation by P388AD is H-2 restricted in that TGG is presented preferentially to primed syngeneic DBA/2 T cells, but not to primed allogeneic C3H T cells. Experiments are underway to determine if additional Ags under Ir gene control can be presented by P388AD tumor cells. Supported in part by NIH grants CA28308, T32 CA09210 and NFCR grant 31362.

5

INABILITY OF UREMIC MACROPHAGES TO PRESENT ANTIGEN TO CONTROL IMMUNE T CELLS. YAEL G. ALEVY*, KATHLEEN R. MUELLER*, AND RAYMOND G. SLAVIN, St. Louis University School of Medicine, St. Louis, MO 63104

The effect of experimentally induced acute uremia in the rat on the response of immune lymph node (LN) cells to antigens in vitro was investigated. It was found that the response of unfractionated uremic LN cells to PPD and DNP-KLH is severely suppressed as compared to the response of LN cells from sham operated rats. The mechanism behind this suppression was studied by culturing control purified LN T cells in vitro with either control or uremic macrophages (MØ) in the presence of antigen for 96 hrs. When purified control T cells were cultured in the presence of control MØ and antigen a marked proliferative response was observed. However when purified control T cells were cultured with uremic MØ and antigen the proliferative response was significantly suppressed. To determine whether uremic MØ are unable to present antigen to T cells, both control and uremic MØ were pulsed with antigen for 3 hrs, washed and added to control T cells. Our results indicate that control antigen-pulsed MØ can present antigen to T cells while uremic antigen-pulsed MØ are unable to do so resulting in a suppressed response. The inability of uremic MØ to present antigen to control T cells may have relevance to the severely suppressed cell mediated immunity and the increased rate of infections in humans with renal failure.

6

IMMUNOLOGICAL STUDIES IN YOUNG HOMOSEXUAL MALES. P.W.A. MANSELL, M.D., J.M. REUBEN, M.D., G.R. NEWELL, M.D., E.M. HERSH, M.D. Department of Cancer Prevention and Department of Clinical Immunology and Biological Therapy, UTSCC M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030

Sixty-seven homosexual subjects at "high risk" of developing either Kaposi's sarcoma or an opportunistic infection with a performance status of 0-1 have been studied immunologically, compared to a heterosexual control group. Studies included T cell subsets, delayed hypersensitivity responses to five skin test antigens, lymphocyte blastogenic responses, NK and suppressor cell activity, ADCC, monocyte adherence, serum lysozyme and serum thymosin α_1. Anti-CMV and EBV titers, standard hematological and biochemical parameters have also been measured. A questionnaire dealing with lifestyle, previous exposure to sexually transmitted diseases and drug use was answered. Five homosexual patients with Kaposi's sarcoma were also studied, two of whom had Pneumocystis carinii pneumonia. Half of the sixty-seven subjects had experienced a variety of infectious diseases or a syndrome of fever, lymphadenopathy, and weight loss in the preceeding months.

The immunological profile showed an absolute lymphopenia (< 20% peripheral white cells), and monocytopenia (< 5%) with skin test anergy in 35%. PHA responses were low as were total T cells in 45% and helper cells in 79%. The helper suppressor cell ratio was less than 1.0 in 60%. Monocyte adherence was diminished while serum lysozyme was elevated. All subjects tested showed elevated Thymosin α_1 (>793 pg/ml) levels and about 50% had a radiosensitive suppressor cell for the PHA response.

These findings are correlated with clinical status, anti-viral titers and lifestyle factors, an attempt will be made to suggest a hypothesis to explain this syndrome.

7

ENHANCED PRODUCTION OF MURINE INTERFERONγBY T-CELLS GENERATED IN RESPONSE TO BACTERIAL INFECTION. George L. Spitalny, Edward A. Havell*. Trudeau Institute, Saranac Lake, NY 12983.

Spleen cell cultures derived from animals infected 6 days earlier with Listeria monocytogenes, produced 10-20 fold more murine interferonγ (MuIFNγ) than spleen cells from nonimmune mice, in response to stimulation with T-cell mitogens. A striking temporal association was found between the enhanced synthesis of MuIFNγ and development of anti-Listeria immunity, in that both the potential for increased MuIFNγ production and the generation of Listeria-protective T-cells developed and then decayed in unison. Treatment of spleen cells with monoclonal anti-Thy 1.2 plus complement virtually abolished the ability of cells from Listeria-immune mice to synthesize MuIFNγ. The T-cells producing MuIFNγ were found to be more susceptible to complement-mediated lysis with monoclonal anti-Lyt 1.2, than with monoclonal anti-Lyt 2.2. The production of MuIFNγ was not affected by treating spleen cells with anti-IgG antisera or with a monoclonal antibody directed against I-A specificities. MuIFNγ was detected 4 hrs after the beginning of mitogenic stimulation of spleen cell cultures, and peak levels of MuIFNγ were reached by 18 hrs. The IFNs synthesized by mitogen-induced spleen cells derived from Listeria-immune mice were relatively liable at pH 2.0 and neutralized by a rabbit anti-MuIFNγ serum, but not by an antiserum having specificities for MuIFNα and MuIFNβ. The apparent molecular weight of the MuIFNγ, as estimated by molecular seiving on a Biogel P-60 column, was estimated to be 38,000, and the isoelectic point as determined by chromatofocusing was extremely heterogenous, ranging between pH 5.0 and pH 7.0.

8

HUMAN OVIDUCTAL (oM) AND PERITONEAL MACROPHAGES (pM) AS CELLULAR MEDIATORS OF INFERTILITY. A.F. HANEY* AND J.B. WEINBERG. Duke and VA Medical Centers. Durham, NC 27705

We have hypothesized that human peritoneal macrophages, through access to the distal oviduct, might adversely influence fertilization by inteacting with the gametes. We have shown that infertile women with endometriosis have more peritoneal fluid and more pM than do normal fertile women or infertile women without endometriosis, and that pM from endometriosis patients phagocytize and destroy more normal sperm (Am J Ob Gyn, in press, 1982). The present study was designed to determine if the oviducts contain macrophages, and if their numbers correlate with certain disease states and anatomic abnormalities of the female reproductive tract. The 38 patients, having laparotomies for sterilization, infertility, or benign gynecologic conditions, were divided into four groups: (I) fertile with normal oviducts (n=12), (II) infertile with distal oviduct obstruction (n=4), (III) infertile with proximal oviduct obstruction (n=12), and (IV) infertile with endometriosis and normal oviducts (n=12). More than 90% of the oviductal and

peritoneal cells were typical of macrophages: they adhered to plastic, phagocytized latex spheres, had Fc receptors, and contained nonspecific esterase activity. Corroborating our earlier studies, women with endometriosis had high numbers of pM as compared to normal women, while those of grp II and III were intermediate. Women with distal oviductal obstruction had very few oM while women with endometriosis had increased numbers of oM which roughly correlated with the numbers of pM present. The presence of these macrophages in the distal oviduct where fertilization occurs supports the hypothesis that mononuclear phagocytes may inhibit fertility by interacting with gametes.

	Macrophages x 10^5 ±SEM (range)	
grp	peritoneal	oviductal
I	19.9± 3.2(11.0- 37.0)	4.3±1.3(1.0-11.0)
II	68.3±21.2(11.7- 160.0)	0.3±0.1(0.1- 0.4)
III	98.9±23.7(12.0- 278.0)	2.8±0.4(0.6- 7.5)
IV	343.3±91.9(34.0-1050.0)	14.8±2.3(2.0-32.6)

9

INHIBITION OF HUMAN NEUTROPHIL ELASTASE BY HUMAN ALVEOLAR MACROPHAGE LYSATE: PARTIAL *CHARACTERIZATION OF THE INHIBITORY ACTIVITY.* M.S. WALD AND E.J. CAMPBELL. Pulmonary Div., Dept. of Medicine, Washington University at The Jewish Hospital of St. Louis, St. Louis, MO 63110.

Although human alveolar macrophages (AM) release elastase activity when cultured in vitro and contain immunoreactive neutrophil elastase (HLE), lysates of AM contain no elastase activity. While investigating this paradox, we have identified and partially characterized inhibitory activity against HLE which is present in AM lysates (L). L were prepared by sonication and freeze-thawing of AM freshly harvested from non-smokers. L inhibited HLE activity against both ^{14}C-elastin and methoxysuccinyl-ala-ala-pro-val-p-nitroanilide. HLE (100ng) was 50% inhibited by *12.5ug (total protein) L; 50ug L completely inhibited 250ng HLE.* In marked contrast, porcine pancreatic elastase and trypsin were not inhibited by L. Boiling reduced the inhibitory activity of L by 70%. All of the HLE inhibitory activity of L eluted with the void volume when applied to Sephadex G-200. We conclude: 1) L contain inhibitory activity against HLE which is distinct from $alpha_1$-antitrypsin and $alpha_2$-macroglobulin; 2) these data may explain the absence of elastase activity in L; and 3) our results indicate another level of complexity of macrophage involvement in proteolytic tissue injury. Supported by USPHS HL 24265.

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ROLE OF MACROPHAGES IN LOCAL IMMUNOREGULATION IN PREGNANT MICE. G.W. WOOD, L. MANNING*, J. HUNT.* Department of Pathology and Oncology, University of Kansas Medical Center, Kansas City, KS 66103

The three major tissues; uterus, yolk sac and placenta, surrounding developing feti in pregnant outbred (Swiss strain) mice were evaluated for the presence of cells capable of suppressing mitogenic responses to phytohemagglutinin of normal Swiss splenic lymphocytes. Cell suspensions were prepared by collagenase/DNase digestion of separately pooled tissues. All cell suspensions contained the normal complement of appropriate tissue cells plus 10-30% macrophages,<5% lymphocytes and <1% granulocytes. Macrophages were defined by morphology and by expression of specific antigens, FcY receptors, C3 receptors and phagocytosis. Lymphocytes were defined by morphology, expression of T cell antigens and expression of surface IgM. Each of the cell suspensions consistently suppressed mitogenic responses by at least 75% relative to control values. All experiments were controlled for cell density artifact. Specific depletion experiments were possible with uterus and yolk sac cell suspensions. The suppressive effects of the uterus (maternal) and yolk sac (fetal) cells were reversed completely by removal either of FcY receptor positive or adherent cells. The results are interpreted to mean that fetal and maternal macrophages mediate local immunosuppression which may be involved in maintenance of fetal integrity in a potentially hostile maternal immunologic environment. Supported in part by NIH grant HD14065.

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THE EFFECT OF PLASMA FIBRONECTIN-MEDIATED PHAGOCYTOSIS ON SUPEROXIDE PRODUCTION BY INFLAMMATORY NEUTROPHILS AND MACROPHAGES. *P.W. GUDEWICZ, C.M. BUYS*, R. KUMAR* AND* J. MOLNAR. University of Illinois at the Medical Center, Chicago, IL. 60680.

Previously we have reported that plasma fibronectin (PFn) promotes the phagocytosis of gelatin-coated latex beads (g-latex) and zymosan (Z) by inflammatory phagocytes without concomitant stimulation of glucose oxidation or chemiluminescence (JRES Abstr. #75, 1981). The present study extends these observations by examining the effects of PFn-mediated phagocytosis on the generation of superoxide (O_2^-) by inflammatory macrophages (PM) and polymorphonuclear leukocytes (PMNL) derived from rat peritoneal exudates. O_2^- was measured at 37 °C by the superoxide dismutase (SOD)-sensitive reduction of cytochrome c at 550 nm in PMNL suspensions (5 x 10^6 PMNL/flask) and PM monolayers (2 x 10^6 PM/dish). We compared the stimulation of O_2^- production in both cell types during the phagocytosis of either g-latex or Z particles that were opsonized in the presence of 1% fresh rat serum or 14 ug/ml of PFn. The phagocytosis of Z opsonized with either rat serum or PFn resulted in a 7-fold increase above nonphagocytizing controls in O_2^- production by 30 min of incubation in PMNL suspensions. In contrast, the phagocytosis of g-latex opsonized in the presence of either rat serum or PFn did not stimulate O_2^- production. A similar failure to stimulate O_2^- production during the phagocytosis of serum or PFn-opsonized g-latex was also observed using PM monolayers. These results provide direct evidence that the ingestion of gelatin-coated particles opsonized with PFn fails to stimulate the generation of reactive oxygen metabolites by inflammatory phagocytes. (Supported by American Cancer Society grant # CH-233)

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FIBRONECTIN MEDIATED PHAGOCYTOSIS OF DENATURED COLLAGEN (GELATIN) COATED PARTICLES BY ISOLATED RAT KUPFFER CELLS. J.M. Cardarelli*, F.A. Blumenstock, F.J. Rourke*, and T.M. Saba. Department of Physiology, Albany Medical College, Albany, NY 12208.

Plasma fibronectin or cold-insoluble globulin, which is identical to opsonic α_2SB glycoprotein (J.B.C. 253:4287, 1978) is deficient in septic injured patients with host defense failure (Science 201:622, 1978). Kupffer cell phagocytic activity *in vivo* correlates with circulating fibronectin levels. We evaluated the influence of affinity purified plasma fibronectin on the phagocytic uptake of gelatin-coated ^{51}Cr sheep RBCs by isolated Kupffer cells. Rat Kupffer cells were isolated by a sequence of in situ liver perfusion with collagenase, pronase digestion, and metrazamide density gradient centrifugation. Following 24 hr culture of the non-parenchymal cells, 95% of the adherent cells were peroxidase positive and able to bind and phagocytize IgG coated sheep erythrocytes (SRBC), thus identifying the adherent cells as Kupffer cells. By isotopic phagocytic assay and rosetting assay, purified fibronectin enhanced binding and phagocytosis of gelatin-coated particles in a dose-related manner. Fibronectin did not enhance the attachment or ingestion of non-gelatin coated fixed SRBC. A fibronectin co-factor which amplifies the opsonic activity of fibronectin as assessed by liver slice bioassay, also amplified by two-fold the fibronectin mediated attachment of gelatinized SRBC to Kupffer cells. Thus, the isolated Kupffer cell monolayer may be valuable to assess the opsonic activity of fibronectin as modulated by co-factors and potentially influenced by various molecules, i.e. collagen, fibrin, actin and heparin, which avidly bind to fibronectin. (Supported by AI-17635; GM-21447; T32-GM-07033 and T32-HL-07194)

13

TUFTSIN RESTORES THE DEPRESSED RETICULOENDOTHELIAL (RE) FUNCTION AFTER SPLENECTOMY (SPLY) AND IMPROVES SURVIVAL FOLLOWING SPLY AND SEPSIS. I.H. CHAUDRY. Department of Surgery, Yale University School of Medicine, New Haven, CT 06510.

Following SPLY there is increased retention in the lung of injected particulate matter and an increased mortality from sepsis in experimental animals. Since tuftsin deficiency exists following SPLY, the present study was conducted to determine if administration of tuftsin would decrease the abnormal pulmonary retention of particulate matter following SPLY and improve survival following SPLY and sepsis. Three groups of rats were studied: Group I - Sham, Group II - SPLY and Group III - SPLY + tuftsin. At 1 or 8 days after sham-operation or SPLY, 50μg tuftsin in 0.5ml H_2O, pH 7.3, or saline was injected i.p. 30 min later, RE function was determined by using ^{131}I lipid emulsion. The liver retention (% injected dose/total organ, mean $\pm$ S.E.) in Group I was 63.9 $\pm$ 3.6, in Group II 34.0 $\pm$ 1.6 and 51.2 $\pm$ 5.0 and in Group III 58.6 $\pm$ 2.3 and 69.0 $\pm$ 1.5 at 1 and 8 days, respectively, while pulmonary retention in Group I was 1.03 $\pm$ 0.08, in Group II 21.5 $\pm$ 2.9 and 17.0 $\pm$ 3.5 and in Group III 1.6 $\pm$ 0.1 and 0.9 $\pm$ 0.04, respectively. Survival following sepsis was studied in

other animals from Group I, II and III. At 7 days post sham-operation or SPLY, the cecum was ligated and punctured (CLP) and removed 16 hrs later (CR). Multiple 200μg doses of tuftsin or saline were administered at the time of CLP, CR, 8 hrs and 16 hrs following CR. 24 hrs after CR, survival rates in the 3 groups were 75% (15/20), 48% (10/21) and 82% (18/22), respectively (Group III $p<0.025$ compared to Group II). Since administration of tuftsin following SPLY restored RE function and improved survival of animals following SPLY and sepsis, tuftsin treatment bears promise as an immunostimulating agent and as prophylaxis against sepsis following SPLY.

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PANCREATIC HORMONAL INFLUENCE OF KUPFFER CELL PHAGOCYTOSIS IN VIVO AND IN THE ISOLATED PERFUSED LIVERS OF RATS. R.P. CORNELL, Division of Science, Northeast Missouri State University, Kirksville, MO 63501.

Experiments were conducted to determine the influence of insulin, glucagon, and somatostatin of Kupffer cell phagocytosis both in vivo and in the isolated perfused livers of rats. Chronic 2-week pancreatic hormonal treatment consisted of twice daily injections sc of NPH insulin with doses ranging from 0.75 U on day 1 to 9.0 U on day 13 and unchanged doses of glucagon (200 μg) and somatostatin (50 μg). Chronic treatment with insulin significantly depressed by 94% intravascular phagocytosis of colloidal carbon administered iv at a dose of 8 mg/ 100 g while glucagon and somatostatin stimulated macrophage endocytic function by 24 and 20%, respectively, compared to the control group half-time of 11.9 ± 0.35 min. Acute treatment with the three pancreatic hormones ip at 30 min prior to carbon administration similarly produced a 29% phagocytic depression after 175 μg of crystalline insulin as well as a 20 and 18% phagocytic stimulation after 200 μg of glucagon and 100 μg of somatostatin, respectively. Administration of D-glucose (600 mg ip) together with the 175 μg dose of insulin prevented hypoglycemia at 30 min but not RES hypophagocytosis suggesting that insulin suppression of endocytic function is not dependent on the lack of glucose availability in plasma. Addition of the three hormones at near physiologic concentrations (20 ng/ml for insulin, 10 ng/ml for glucagon, and 5 ng/ml for somatostatin) to the recirculating perfusate of isolated perfused rat livers simultaneous with 24 mg of colloidal carbon likewise resulted in a 19% reduction after insulin and a 14% enhancement after both glucagon and somatostatin of Kupffer cell phagocytosis compared to the control group half-time of 40.2 ± 1.83 min. (Supported by DCWA of St. Louis and AM-22102).

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IN VITRO ASSAY FOR MACROPHAGE MOVEMENT. M.A.LEYKO*(Introduced by:Michel Rabinovitch) Institute of Environmental Medicine, New York University Medical Center, N.Y., N.Y. 10016.

An in vitro movement assay for pulmonary alveolar macrophages(PAM)has been devised. This method is an adaptation of the Albrecht-Buehler technique (1976). It involves preparing a dense and even layer of 0.5μ latex particles which adhere to a plastic substrate via a thin coat of bovine serum albumin. PAM's are seeded on this particle coated substrate and with time they engulf and ingest the particles leaving behind a cleared path. The particle-free tracks provide a record of the cells migratory activity. Turning behavior, the number of cells that move as well as the average area of cell movement can be determined. This method has been used extensively with rabbit PAM's but mouse PAM's and peritoneal exudate cells (PEC) are also active. Exposure of rabbit PAM's to chemoattractants such as Nor-Leu-Leu-Phe (10^{-6}M) show increased activity. Movement is blocked by addition of cytochalasin B (10^{-5}M). Response of macrophages agrees with information obtained from other methods. Currently the method is being used to monitor rabbit PAM's exposed in vivo to magnetite, an inert dust, or a cytotoxic tin mine dust from the People's Republic of China.

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MACROPHAGE-DERIVED CHEMOTAXINS: ROLE IN THE LUNG'S INFLAMMATORY RESPONSE TO INTRATRACHEAL IRON OXIDE. J. Dauber, E. Lugano‡ R. Daniele. Univ. of Pennsylvania, Pa. 19104.

Chemotaxins derived from alveolar macrophages (MDCF) appear to mediate inflammation in acute experimental silicosis (Am Rev Resp Dis 125(4,Pt2):149, 1982). Here

we examined the role of MDCF in the inflammatory response to the intratracheal injection of Fe_2O_3, a non-toxic particle. Guinea pigs were sacrificed 1,2,4,7 and 14 days after injection of 50mg of Fe_2O_3 or saline. Cells in lavage fluid were enumerated, adherent macrophages cultured for 2 hours and culture medium assayed for chemotactic activity. Lungs were also prepared for histology. Fe_2O_3 provoked an inflammatory response around terminal bronchioles. Neutrophils (PMN) predominated initially but quickly gave way to macrophages (AM). By day 14, infiltrates had nearly resolved though iron-laden cells were still evident. An increased number of PMN, AM, eosinophils and lymphocytes were recovered by lavage on days 1,2,4. The increase was greatest for neutrophils (20 x control on day 1). By day 7, recovery was normal. Chemotactic activity for PMN and peritoneal macrophages in experimental cultures was comparable to the level in control cultures for only day 2 animals. It was negligible and significantly less than in corresponding controls for day 1,4,7, 14 animals. Thus, iron oxide, as opposed to quartz, provokes a reversible inflammatory response in the lung. The role of MDCF in initiating this response is questionable, and it is possible that AM exposed to Fe_2O_3 *in vivo* contribute to the suppression of associated inflammation. (Supported by NIH Grants HL 23877 and HL 06230).

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DEACTIVATION OF ALVEOLAR MACROPHAGE RESPONSE TO CHEMOTACTIC FACTORS. C.C. Daughaday, J. Mehta*, A.N. Bohrer*, and I. Spilberg*. V.A. Medical Center and Washington University, St. Louis, MO 63125.

Deactivation is the loss of chemotactic responsiveness following brief exposure to a chemotactic factor. Alveolar macrophages (AM) produce superoxide anion and demonstrate a chemotactic response to N-formyl-methionyl-leucyl-phenylalanine (FMLP) and C5a fr, but do not release lysosomal enzymes during short incubation with either chemotaxin. Therefore, we studied the deactivation of guinea pig AM obtained by tracheal lavage. After incubation with FMLP or C5a fr for 30 minutes, chemotaxis was assayed in blind well chambers. Preincubation of AM with 10^{-6}M FMLP resulted in 84% deactivation of the response to FMLP and 22% cross-deactivation of the response to C5a fr. Preincubation of AM with C5a fr (100 ul/ml) resulted in 68% deactivation of the response to C5a fr and 33% cross-deactivation of the response to FMLP. Deactivation, but no cross deactivation, was observed when AM were preincubated with lower concentrations of FMLP (10^{-8}M) or C5a (10 ul/ml). Parallel studies of superoxide anion production showed an increase in the baseline and inhibition of the incremental response to FMLP and C5a fr following preincubation with either chemotaxin. We conclude that AM deactivation is relatively stimulus specific but cross-deactivation occurs with high concentrations of chemotaxins, suggesting that the phenomenon of deactivation in the AM is primarily a post-receptor event where degradation by granular enzymes does not appear to play a role.

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THE FATE OF *PASTEURELLA HEMOLYTICA* IN NORMAL MOUSE PERITONEAL AND ALVEOLAR MACROPHAGES. F.M. COLLINS, S.S. MTERO, C.J. NIEDERBUHL AND S.G. CAMPBELL. Trudeau Institute, Saranac Lake, N.Y. and Cornell University Veterinary College, Ithaca, N.Y.

P. hemolytica causes a fatal fibrinous pneumonia in cattle but is avirulent for mice whether introduced by the I.V., I.P. or aerogenic routes. Viable counts carried out on the lungs of aerogenically challenged mice showed rapid inactivation of the bacterial population in the lung with no systemic spread to the liver and spleen. An intraperiteneal challenge was killed with no sign of extracellular growth or the peritonitis characteristic of *P. multocida* infections. Inactivation was not affected by prior whole-body irradiation (600-900 R). *P. hemolytica* established a persistent intestinal infection in germ-free ICR mice, with as many as 10^{10} viable bacilli recovered from the gut contents by day 4. The ileal Peyer's patches and the mesenteric lymph node became heavily infected (10^7 CFU by day 7). However the lungs, liver and spleen remained uninfected throughout the experiment. None of these animals died. Attempts to demonstrate an inhibitory factor in mouse serum or lung homogenate were negative. Mouse periteneal (PEC) and alveolar macrophage (AM) monolayers were infected *in vitro* with *P. hemolytica*. The rate of kill was compared to that seen for a standard *L. monocytogens* suspension. Unlike bovine alveolar macrophages, mouse AM were not inactivated by toxic factor(s) released by the *P. hemolytica*. Mouse macrophages were only poorly phagocytic for non-opsonized *P. hemolytica*; never-the-less substantial intracellular killing was observed. Possible reasons for the lack of mouse virulence shown by this important bovine pathogen will be discussed.

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CELLULAR AND SERUM ALTERATIONS INDUCED BY CHRONIC PSEUDOMONAS PULMONARY INFECTION. G. WINNIE,* J. KLINGER,* P. CHENG,* M.J. THOMASSEN. Case Western Reserve University, Cleveland, Ohio 44106.

Chronic Pseudomonas aeruginosa (PA) pulmonary infection in cats results in 1) the development of a specific phagocytic inhibitory activity in the serum and 2) sequential changes in the lung cell population. The chronic pulmonary infection was established in the right lower lobe by the injection (using a fiberoptic bronchoscope) of PA (strain JR-1, serotype 5) enmeshed in agar beads, and maintained by reinjection every 2 weeks for up to 60 weeks. Lung cells were obtained by lavage using normal saline and the bronchoscope. Phagocytosis by alveolar macrophages (AM) was measured by incubating AM from uninfected cats as monolayers for 20 minutes in the presence of ^{3}H-bacteria and 5% serum from control or infected cats. By 16 weeks after initial infection, sera from 4 infected animals inhibited phagocytosis of PA strain JR-1 and an additional PA strain (serotype 5). However, phagocytosis was enhanced by these sera when tested with PA serotype 6. Phagocytosis of Serratia marcescens and Staphylococcus aureus was unchanged. Inhibition of phagocytosis (31-71%) was decreased to almost control levels by preadsorption of serum with strain JR-1. Gel filtration of serum on Sephacryl-200 demonstrated the inhibitory activity to be associated with the IgG fraction. The percentage of AM in lavage fluid from infected animals decreased from 74 ± 7% before infection to 25 ± 9% six weeks after initial infection and remained depressed, while the percentage of AM recovered from an animal inoculated with sterile beads remained greater than 80%. Macrophages from infected animals appeared larger, with increased membrane ruffling, lysozomes, and phagocytic ability. Thus, alterations in both systemic and pulmonary immunologic responses occur as a result of chronic PA pulmonary infection.

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FREQUENCY OF BLACK PIGMENT IN LIVER AND SPLEEN OF COAL WORKERS. M.E. LEFEVRE, F.H.Y. GREEN*, D.D. JOEL, W. LAQUEUR*. Medical Research Center, Brookhaven National Lab., Upton, NY 11973 and Pathology Section, NIOSH, Morgantown, WV 26505.

This study sought to provide information on the degree to which coal-mine dust becomes systemically distributed and retained in exposed workers. Histological sections of liver and spleen taken at autopsy from 98 retired coal workers and 9 non-coal workers were scored for black pigment. Pigment was minimal in the non-coal workers. Livers and spleens of the majority of coal workers were free of large deposits of black pigment; however, moderate or heavy pigment was seen in 10.4% of liver and 19.5% of spleen sections from coal workers. Evidence that the pigment was coal-mine dust will be presented. Information on pulmonary pathology and occupational exposure to mine dust was available for most of the workers. Highly significant positive correlation was found between severity of pneumoconiosis and black pigment score in the case of both liver ($p<.001$) and spleen ($p<.001$). Significant positive correlations were found between years underground ($p<.01$), years of retirement ($p<.05$), and age at death ($p<.005$) vs pigment score. Significant negative correlation was found between smoking and pigment ($p<.05$). Severity of pneumoconiosis in coal workers is known to be related to exposure history and to the amount of coal-mine dust within lungs. The positive association of black pigment with age at death, years of underground mining, and severity of pneumoconiosis indicates that cumulative lifetime exposure plays a role in dust accumulation in liver and spleen. The implications of the findings for questions on the sequestration of dust within lungs, modes of migration from lungs, and long-term retention in liver and spleen will be discussed.

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RAT MACROPHAGES REQUIRE Ia ANTIGEN TO RELEASE LEUKOCYTIC ENDOGENOUS MEDIATOR (INTERLEUKIN -1). R. H. MITCHELL, N. J. LOVING*, and R. F. KAMPSCHMIDT. Biomedical Division, Samuel Roberts Noble Foundation, Ardmore, Oklahoma 73401.

Macrophages (Mϕ) release a monokine, Leukocytic endogenous mediator (LEM), which causes neutrophil (PMN) release, changes levels of plasma metals and acute phase proteins, and stimulates thymocyte proliferation (LAF). The need for Ia antigen on rat Mϕ to release LEM (IL-1) was studied.

Three day proteose peptone elicited peritoneal Mϕ (PPMϕ) were compared to 3 day 10^6 live *Listeria monocytogenes* elicited peritoneal macrophages (LPMϕ) for ability to release LEM into the supernatant when incubated overnight on RPMI 1640. PPMϕ (low % Ia+) or LPMϕ (high % Ia+) released neither LAF activity [375 ± 32 (13) and 609 ± 51 (25) dpm ^{3}H-TdR] nor PMN-releasing activity [2718 ± 422 (6) and 2265 ± 365 (21) PMN/mm^3] without further stimulation. When pulsed 1 hr with 1μg/ml *Salmonella abortus equi* endotoxin LPMϕ released LAF and PMN activity [3019 ± 439 (25) dpm, $p < .001$; 5923 ± 488 (23) PMN/mm^3, $p < .001$] whereas PPMϕ did not [942 ± 177 (13) dpm; 3136 ± 396 (5) PMN/mm^3]. Flatbed isolectric focusing of supernatant from LPMϕ pulsed w/endotoxin showed a primary peak of both LAF and PMN-releasing activities with a pI of pH 5.5 with a possible second peak at pH 5.1. Neither LAF nor PMN-releasing activity was found in the pH 7 - 8 range. Presence of the Ia antigen on rat peritoneal Mϕ appears to be necessary to allow further acitvation to release LEM (IL-1).

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Potentiation of Allograft Responsiveness by Plasma Fibronection. D. B. Lause[1], J. E. Doran[2,3], J. A. Houston[1] and D. H. Beezhold[1]. Departments of Anatomy[1] and Surgery[2], Medical College of Georgia, Augusta, GA 30912 and the Veteran's Administration Medical Research Service[3], Augusta, GA 30910.

Recent evidence from this laboratory has shown that plasma fibronectin (Fn) exerts an inhibitory effect on lymphoproliferation to mitogens and alloantigens. However, our recent work employing purified cell populations has demonstrated a potentiating effect for Fn in the mixed lymphocyte response (MLR). We have established the experimental requirements which demonstrate a stimulatory role in the rat MLR for splenic macrophages purified in flat-bottomed microtiter plates. Target cells were 94% phagocytic as evidenced by their uptake of carbonyl iron particles. Incubation of 50 μg of Fn with optimal concentrations of irradiated (DA x Fisher) F_1 macrophage stimulators and unseparated or nylon wool purified DA lymph node cell (LNC) responders resulted in a significant enhancement of the MLR when compared to the MLR in the absence of Fn and the syngeneic MLR in the presence of Fn. Fn generally had no potentiating effect on the syngeneic MLR. Optimal proliferative activity for the MLR with or without Fn occurred on day 5 of incubation. Enhancement of proliferation by Fn occurred as early as 72 hr in culture. High concentrations of macrophage stimulators in the presence of Fn failed to enhance the MLR and in some cases evoked inhibition of the response. Due to the purity of the cell populations, this experimental approach to the MLR provides for a highly efficient interaction between responders and targets. Fn, which has been shown to bind to macrophages, may increase this efficiency by stabilization of the lymphocyte-macrophage interaction. (Supported by NIAID grant 1948401).

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HUMAN MONOCYTE MIGRATION INHIBITION (MMI) BY HUMAN INTERFERON (IFN) AND INTERFERON-INDUCERS. GARY B. THURMAN AND HENRY C. STEVENSON*. Biological Response Modifiers Program, National Cancer Institute-FCRF, Frederick, MD 21701.

Partially purified human α, β, and γ IFN are potent inhibitors of the migration of monocytes from normal human volunteers. Using the agarose droplet technique, with monocytes purified by Ficoll-Hypaque gradients followed by centrifugal elutriation, we found that purified recombinant leukocyte (α) IFN is also potent in its MMI ability. MMI activity has been evident at IFN concentrations as low as 10 IU/ml, with γ IFN appearing to have the most MMI activity per unit of anti-viral activity. IFN-inducing agents such as poly I:C are also effective in MMI at concentrations that are non-toxic. These observations raise a note of caution in ascribing migration inhibitory factor (MIF) activity to antigen or mitogen-induced supernatants that may also contain IFN or IFN-inducing agents. Supernatants from cell lines reported to make MIF must also be evaluated for IFN activity since the MIF assay does not distinguish between MIF and IFN. IFN appears to act directly on macrophages to inhibit their migration, since a human macrophage-like cell line, U937, was also inhibited from migrating by IFN. The mechanism by which IFN inhibits macrophage migration is not known, but our results raise the possibility that macrophage MIF may act through the induction of IFN production, perhaps by the macrophages themselves, which in turn limits cellular migration.

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THE STUDY OF A MONOKINE THAT ENHANCES LYMPHOCYTE MIGRATION IN VITRO. H.T. CHEUNG AND J.S. TWU. Department of Biological Sciences, Illinois State University, Normal, IL 61761.

The culture supernatants of mouse peritoneal exudate macrophages (PEM) contained a monokine that enhanced the in vitro migration of spleen lymphocytes. This lymphocyte migration enhancement factor (LMEF) was found in the culture supernatants of non induced PEM and PEM induced by complete Freund's adjuvant and thioglycollate. The treatment of PEM with lipopolysaccharide in vitro, however, slightly enhanced the LMEF in the culture supernatants. The effect of the LMEF was neither strain nor species specific because the LMEF produced by PEM obtained from C57BL/10 mice was equally effective on the migration of lymphocytes of B10.G and BALB/c mice and Fischer F344 rats. The maximum LMEF activity was found in culture concentration of 1×10^6 PEM/ml, and the activity was detectable after an eightfold dilution with culture media. Addition of indomethacin to PEM during culture did not inhibit the production of LMEF activity, which suggests that the LMEF is not a prostaglandin. Furthermore, in ultrafiltration studies using Amicon filters, the molecular weight of LMEF was determined to be approximately 7,000 daltons. We propose that the LMEF enhances lymphocyte locomotion in vivo to increase the interaction of lymphocytes with macrophages and that the LMEF modulates lymphocyte migration through lymphoid organs.

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INHIBITION OF IN VITRO LYMPHOCYTE RESPONSES BY VERY LOW DENSITY LIPOPROTEINS FROM DIABETIC RAT SERUM. D.S. CHI, D.L. BERRY*, K.A. DILLON*, AND B.W. ARBOGAST*. Department of Internal Medicine, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, TN 37614.

Immune function is generally reported to be impaired in uncontrolled diabetes mellitus. Very low density lipoproteins (VLDL) have recently been found to be toxic to aortic endothelial cells in culture and we wanted to determine if VLDL also injured lymphocytes and impaired the immunity of the streptozotocin-induced diabetic rat. In diabetic rats peripheral blood (PB) leukocytes, spleen cells and the percentage of PB lymphocytes were significantly decreased ($p < 0.02$). Mitogenic stimulation responses of spleen cells from diabetic rats were consistently lower than normal for phytohemagglutin (PHA), pokeweed mitogen and concanavalin A. These decreased responses were not due to the induction of suppressor cells. The effects of diabetic serum on mitogenic responses of normal lymphocytes were studied. Diabetic rat serum inhibited the proliferation of mitogen stimulated normal rat lymphocytes. Addition of increasing amounts of VLDL, isolates from diabetic serum, to normal spleen cell cultures also resulted in an exponential decrease in ^{3}H-thymidine incorporation. Complete inhibition of the response of normal spleen cells to PHA was achieved at a VLDL triglyceride concentration of 262 µg/ml. The 4 day old lymphocyte cultures incubated with VLDL at 262 µg/ml concentration were only 11% viable which was significantly lower than the 58% viability of control cultures without VLDL ($p < 0.005$). These data suggest that elevated levels of VLDL may impair the host defense through a toxic effect on lymphocytes.

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CHARACTERIZATION OF ALANINE AND TYROSINE AMINOPEPTIDASE ACTIVITIES IN LUNG MACROPHAGES. G.A. WARR, P.L. SANNES*. Department of Environmental Health Sciences, the Johns Hopkins University, Baltimore, MD 21205.

Aminopeptidases offer the phagocytes an enzymic mechanism whereby the cells can modify numerous proteins. These protein modifications may drastically alter the biological potential of the molecule. This study reports on the enzymic characteristics and localization of two aminopeptidases, using synthetic L-alanyl- and L-tyrosyl-2-naphthylamide (Ala-NA & Tyr-NA) substrates. The enzymic product (2-NA) was coupled with fast garnet, and quantitated spectrophotometrically at 520nm. Rat lung macrophages (Mø) were studied in vitro as viable cell and Mø-lysate preparations. The enzymic pH optimum was 7.0-7.5, and activity was linear with both cell number and incubation time. The hydrolysis of Tyr-NA by viable Mø was significantly ($P < 0.001$) less than the Ala-NA (33.8±3.8% Ala-NA). Using Michaelis-Menton analysis, viable Mø hydrolysis of Ala-NA had an apparent Km of 0.13mM and a Vm of 186uM hr^{-1}. The enzymic activity of Mø-lysates was significantly lower (51.3±1% viable Mø), and the Km was increased to 0.32mM with a Vm of 91uM hr^{-1}. Viable Mø hydrolysis of Tyr-NA had an apparent Km of 0.18mM and a Vm of 104uM hr^{-1}. After lysis, the Km was 0.25 mM and

the Vm 96uM hr^{-1}. Since the activities were not increased with Mø-lysis, the enzymes might be exposed on the cell surfaces. To test this hypothesis, viable Mø were incubated with diazotized sulfanilic acid or p-chloromercuriphenylsulfonic acid. Both surface-active compounds inhibited aminopeptidase activities. Electron microscopic cytochemistry confirmed the aminopeptidases were ecto-enzymes. Thus, these enzymes are in a position to interact with external proteins contacting the Mø, and may be important for the regulatory/functional activities of the cell.

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PARAMETERS EFFECTING HEPATIC CLEARANCE AND KILLING OF MICROBES. R. T. SAWYER, E. J. CABRERA,* K. J. NEUMANN,* M. RAVELI,* Mercer University School of Medicine, Macon, GA, Norwich-Eaton Pharmaceuticals, Norwich, NY, East Carolina University School of Medicine, Greenville, NC.

Parameters effecting the ability of CD-1, C57Bl/6N and beige mice to clear and kill microbes from deep tissues were studied using the perfused liver model. The ability of perfused hepatic tissue from these mice to trap and kill S. typhimurium (SR11), P. aeruginosa (PS44) and L. monocytogenes (L12) was evaluated using various perfusion media and serum components. In the absence of serum CD-1 hepatic tissue trapped 61% of 10^6 PS44 killing 23% and 32% of 10^6 SR11 killing 17% using RPMI 1640. When M199 was used trapping and killing parameters were decreased for PS44 and SR11. Addition of serum to HBSS increased hepatic killing of SR11 in CD-1 mice by 22% ($P<.05$). By contrast, addition of serum or guinea pig complement to RPMI 1640 decreased killing of PS44 in CD-1 mice by 15-23% ($P<.05$). CD-1 hepatic tissue was unable to kill L12 either in the presence or absence of serum factors and trapping occurred in a dose dependent manner. In the presence of serum black mice increased hepatic trapping of SR11 by 27% with 48% killing. In beige mice trapping rose 7% with 18% killing suggesting that this mutation might include a trapping, as well as or in addition to a phagocytic, defect in hepatic tissue. The data suggest that further study is needed to characterize both microbial surface-host tissue interactions in hepatic trapping and killing and possible genetic defects in this component of microbial vascular clearance. (Supported in part by the United Way of NC)

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NON-SPECIFIC MITOGENIC RESPONSES OF LEW AND F344 RAT LYMPHOCYTES TOWARD MYCOPLASMA PULMONIS. J.K. Davis*, J.W. Simecka*, R.B. Thorp*, and G.H. Cassell. Univ. Ala. Birmingham, Birmingham, AL 35294.

M. pulmonis mitogenicity has been suggested to play a role in the pathogenesis of M. pulmonis respiratory disease. LEW rats are more susceptible than F344 rats and show a greater increase in the number of lung lymphocytes in vivo. To determine if there is a difference in the non-specific mitogenic responses of LEW and F344 lymphocytes toward M. pulmonis and to determine if this was limited strictly to M. pulmonis, spleen and lung lymphocyte cultures, containing autologous serum, were exposed to various doses of M. pulmonis mitogen, PHA and PWM. LEW spleen and lung lymphocytes responded significantly higher ($p<0.001$) to all three mitogens (LEW to F344 ratio at maximum stimulating dose ~ 2:1, 1.5:1, and 4:1 for M. pulmonis, PWM and PHA); even though cultures from the two strains were essentially identical in lymphocyte to macrophage ratios and in T to B lymphocyte ratios. The difference between LEW and F344 spleen lymphocyte responses was not entirely due to differences in LEW and F344 sera used in the cultures as switching sera had little effect on the magnitude of response. When LEW and F344 cells were mixed, the lymphocyte response to all three mitogens was signicantly higher than expected. Thus, the difference in disease susceptibility seems to be due primarily to differences in regulatory mechanisms. (Supported by NIH grant HL 19741).

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Lysosomal enzymes (LE) and microbicidal capacities of alveolar macrophages in pulmonary defense mechanisms. S. Chandrasekhar and M.K. Mukerji, Department of Microbiology, V.P. Chest Institute,

University of Delhi, Delhi-110007(India). Acquired resistance to tuberculosis is essentially the result of intracellular bacteriostasis in which digestive hydrolases of the lysosomes are considered to play an important role. Increased amounts of LH have been demonstrated in activated or immune macrophages. If this is so, a relationship should exist between the number of bacilli inside a cell and its lysosomal enzyme contents. This study is an attempt to evaluate the viability of such a concept. Tubercle bacilli were injected intratracheally into normal and BCG vaccinated guinea pigs. Alveolar macrophages were collected and their lysosomal enzymes estimated histochemically along with the number of acid fast bacilli present inside the cell. Following enzymes were studied. Superoxide dismutase, β-galactosidase, Lipase, N-acetyl glucosaminidase and lysozyme. It was found that the activity of lysosomal hydrolases though increased in immune animal did not proportionately affect the number of bacilli inside the macrophage.

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PROGRESS IN THE PURIFICATION AND CHARACTERIZATION OF MACROPHAGE CYTOTOXIN (MCT). J. KLOSTERGAARD*, T.H. REIDARSON* AND G.A. GRANGER. Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, CA 92717.

Exposure of thioglycollate-elicited peritoneal exudate macrophages (PEM) to poly-I:C or LPS, or of alloimmune PEM to tumor cells or poly-I:C or LPS, induces the release of a macrophage cytotoxin (MCT). MCT is toxic for a variety of syngeneic and allogeneic tumor cells, and shows no effect on normal cells in vitro. The toxin has the characteristics of a neutral protease of trypsin-like specificity. Molecular sieving of the concentrated, serum-free, MCT-containing supernatants on Ultrogel AcA 44 reveals a molecular weight of 150,000 for the major MCT moiety. Employing radioiodinated preparations of sieved MCT, ion-exchange chromatography on DEAE-Sepharose or on phosphocellulose, or lectin-affinity chromatography on Con A-Sepharose or on PHA-Ultrogel, are unable to significantly purify the toxin. The labile toxin cannot be recovered in a lytically active form from preparative isoelectric-focusing in sucrose or from gels after PAGE in the absence of $NaDodSO_4$. Very significant purification can be realized by a combination of negative and positive hydrophobic affinity chromatography on a tandem of aryl and alkyl sorbents. The highly purified MCT will be used to develop a specific antiserum to probe the possible role of MCT in direct macrophage-mediated tumoricidal reaction in vitro.

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SIMULTANEOUS MEASUREMENTS OF CYTOSTASIS AND CYTOTOXICITY USING THE FLOW CYTOMETER. A.P. STEVENSON, J. C. MARTIN AND C.C. STEWART. Los Alamos National Laboratory, Experimental Pathology, Los Alamos, NM 87545

We have used flow cytometry to study the kinetics of EMT6 tumor cell growth following exposure to tumoricidal peritoneal macrophages from BCG-injected C57BL/6. At 24-h intervals tumor cells were trypsinized and stained with the fluorescent dye Hoechst 33342. Cells were anlayzed for DNA content and relative volume using a Los Alamos-developed flow cytometer. Macrophages were resolved from tumor cells by their lower DNA content. Thus, by adding to each cell suspension fluorescent latex particles whose concentration is known, estimates were obtained of the actual number of EMT6 cells present, and these were compared to the number in cultures containing either non-tumoricidal macrophages or no macrophages. The percentage of EMT6 cells in each phase of the cell cycle was also determined. In order to estimate the relative contributions of cytostasis and cytotoxicity to the observed decreases in growth rate and changes in cell cycle distribution, the percentage of cells in S phase was compared to the labelling index as a function of time after incubation with the tumoricidal macrophages. In addition, the degree to which tumor cell growth was inhibited as a function of macrophage concentration was studied during the first 24 h. The results showed that exposure of EMT6 cells to tumoricidal macrophages resulted in an absolute decrease in survival as well as changes in cell cycle distribution. These cell cycle changes appear to reflect both cytostasis and cytocidal activity. This work was performed under the auspices of the U.S. Department of Energy at the National Flow Cytometry Resource and was supported by Grant #CA33593 awarded by NCI.

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DIFFERENTIAL CYTOTOXIC EFFECTS OF PERITONEAL MACROPHAGES AND THE J774 MONOCYTIC CELL LINE ON METASTATIC VARIANTS OF MURINE B16 MELANOMA AND RAW 117 LYMPHOSARCOMA IN VITRO. K.M. MINER,* J. KLOSTERGAARD,* G.A. GRANGER, and G.L. NICOLSON. (Introduced by: P. Davies). Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717, and Department of Tumor Biology, M.D. Anderson Hospital, Houston, Texas 77030.

The cytolytic and cytostatic effects of murine peritoneal macrophages (PM) and J774 reticulum cell sarcoma cells against various metastatic variants of murine tumors was investigated in vitro. Thioglycollate elicited PM or J774 cells were plated at different densities in growth medium plus fetal calf serum for two hours. The cells were then washed and activated in vitro by a one-hour exposure of polyinosinic:polycyticylic acid (poly I:C) in growth medium plus 0.01% lactalbumin hydrolysate as a serum substitute. Tumor cells were added to the activated macrophage monolayers and direct cell cytotoxicity was determined using one of two methods. A) For adherent B16 melanoma variants, a 48 hour radioactive release assay using ^{125}IUdR prelabeled targets measured cytolysis, and B) for non-adherent RAW 117 lymphosarcoma variants, cytolysis and cytostatis was determined by counting non-dividing (mitomyocin-C treated) or dividing target cells after a 40 hour incubation. Our results indicate that high metastatic tumor cell variants from these two tumor systems are more resistant to in vitro cytolysis and cytostasis by poly I:C activated PM than low metastatic variants. Furthermore, cells from the J774 macrophage-like cell line behaved in a manner similar to that of PM suggesting that this cell line may be a suitable model with which to study the differental cytolytic effects observed among metastatic variants. This work suggests that the high metastatic potential of certain tumor cells may, in part, be due to their ability to circumvent destruction by activated macrophages.

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DISTINCT REGULATORY MECHANISMS FOR ANTIBODY-DEPENDENT (ADCC) AND NONSPECIFIC KILLING OF TUMOR TARGETS BY MURINE AND HUMAN MACROPHAGES, AND ROLE OF Ig CLASS IN ADCC. P. Ralph, I. Nakoinz*, N. Williams*, C. Punjabi*, P. Litcofsky* Sloan-Kettering Institute for Cancer Research, Rye, NY 10580.

Pretreatment of murine resident peritoneal or RAW264.10A line macrophages with LPS 1 μg/ml, 0.1 μg/ml tumor promoter TPA, lymphokine (LK) or drugs inhibiting microtubule function (1 μM taxol, 0.5 mM lidocaine, 10(-7)M colchicine) greatly stimulated ADCC to myeloid and lymphoid tumors, but had no effect or inhibited cytotoxicity to the same targets induced by LPS or TPA. LK (MAF) induced strong killing of sarcoma but not lymphoma targets without antibody. Stimulation of ADCC factor (SAF) was present in some preparations lacking MAF. (In LK, SAF was 60,000 mw and distinct from MAF and myeloid colony-stimulating factors, experiments with C.A.Nacy and M.S. Meltzer.) SAF was heat sensitive in contrast to LPS. Human monocyte line U937 was induced for ADCC by 5 day treatment with human LK, 0.1 μg/ml TPA, or cytokine from a carcinoma line. Human SAF was distinct from IL-2, interferon (K. Welte, R. Mertelsmann), CSF or B lymphocyte differentiation factor. Nonspecific tumor killing was also induced in U937, but only by TPA. The pattern of surface antigens induced in U937 and murine M1 cells suggests that certain surface structures are not directly involved in cytotoxicity. Induction of the immature U937 cells to ADCC probably occurs in stages; a 40,000 - 60,000 mw LK is required for induction of Fc receptors but this is not sufficient for cytotoxicity. Human pleural and peritoneal macrophages were stimulated for ADCC similarly to murine cells. Murine monoclonal antibodies of all four IgG classes, but not IgM, directed ADCC to tumor targets.

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THE RELATIONSHIP BETWEEN NATURAL KILLER CELLS AND EFFECTOR CELLS INVOLVED IN LECTIN DEPENDENT CELLULAR CYTOTOXICITY AND ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY IN MAN. T.P. BRADLEY, B. BONAVIDA. UCLA School of Medicine, Los Angeles, California 90024.

Human peripheral blood lymphocytes (PBL) mediate several cytotoxic functions such as natural killing (NK), lectin dependent cytotoxicity (LDCC), and antibody dependent cytotoxicity (ADCC). Contradictory reports have arisen as to whether the same or distinct cells mediate these various cytotoxic functions using the ^{51}Cr-release assay. In this study, a two-target conjugate assay (J.Immunol. 126:208,1981) involving two different targets bound simultaneously to a single effector cell was used to directly resolve the question of uni- versus multipotentiality of effector cells. PBL and Percoll gradient enriched LGL were used as a source of cytotoxic cells. K562 or MOLT were used as NK targets, antibody treated RAJI or P815 for ADCC targets, and Con A, PHA or periodate treated RAJI as LDCC targets. Significant binding of single

cells to two heterotargets was observed in all cases. The results of single cell lysis revealed that the same cell lyses both NK and ADCC targets. However, NK effectors which kill NK targets did not kill ADCC targets. These results unequivocally demonstrate that NK and ADCC cytotoxic reactions in unstimulated PBL are mediated by the same effector cells, whereas the LDCC effector cell is distinct. The multipotential activity of certain effector cells suggests that distinct multiple receptors on the same cell are involved in activating the lytic pathway of cytolysis.

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ALTERED SENSITIVITY TO MACROPHAGE-MEDIATED CYTOTOXICITY IN PRIMARY AND METASTASIS-DERIVED MURINE TUMOUR CELL LINES. F.P. NESTEL*, R.H. WILTROUT* and R.S. KERBEL*, (Introduced by H.F. Pross). Cancer Research Laboratories, Department of Pathology, Queen's University, Kingston, Ont. K7L 3N6.

Murine cell lines derived from the metastatic DBA/2 tumor, MDAY-D2 and selected for their ability to metastasize were examined for sensitivity to lysis by activated macrophages. The plating efficiency of macrophages from C57BL/6 peritoneal exuhate cells (PEC) was 16% as assessed by limiting dilution analysis. A standard curve for the number of cells plated was developed using protein determination on washed monolayers of PECs. This quantitation permits an accurate assessment of the number of effectors plated per well. Cytotoxicity was measured in a 48 hour Indium-III release assay using lymphokine and lipopolysaccharide activated macrophages. The dose response in level of cytotoxicity seen at various concentrations of lymphokine allows different tumour cell lines to be compared for macrophage-mediated lysis. Cloned tumour cell lines derived from primary and metastatic tumours of DBA/2 mice injected with MDW4, a wheat germ agglutinin-resistant variant of MDAY-D2, exhibited marked differences in susceptibility to macrophage mediated cytolysis. The ability of these cell lines to metastasize was not associated with their relative in vitro macrophage sensitivities.

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TIME-RESPONSE CHARACTERISTICS OF THE CELLULAR IMMUNE RESPONSE TO THE H-1 ANTIGEN. M. Kurtz*, D. Martin*, H. Russell*, R.J. Graff* (Introduced by: David Thomasson). Department of Surgery, John Cochran Hospital and Washington University School of Medicine, St. Louis, Missouri 63110.

The cellular response to weak transplantation antigens offers an exceptional model to study the feedback circuitry of the immune response. Previous experiments using the allograft rejection reaction to study the time-response relationships for transplantation antigens of varying strengths have shown the level of response to strong antigens to increase rapidly following first antigen contact. With weak antigens immunity was preceded and followed by periods of tolerance. In order to study the mechanism of this observation we have utilized the CML assay which will allow us to study the composition of lymphocyte populations at various times in the immune response. Female B10-H-1^b mice were immunized by the IP injection of $5x10^6$ B10-H-1^c spleen cells and the level of host immunity at various intervals thereafter was measured using the secondary CML assay. Immunity was shown to appear on day 7, peak on day 10, gradually fall to a low level on day 42 and increase until the last observation on day 56. The slopes of the titration curves on the various days of observation were constant indicating that the changes in immune responsiveness had resulted from changes in the numbers of CTL's rather than in efficiency of the individual cell. Preliminary data indicates a much slower response to the H-3^c antigen. (Research supported by USPHS grant #AI 07437 and the Veterans Administration).

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DIFFERENTIAL ADJUVANT ACTIVATION OF MACROPHAGES FROM YOUNG AND AGING MICE. P.R. PETREQUIN, A.G. JOHNSON. University of Minnesota, Duluth, MN 55812.

Macrophages from young (3-6 months) and aging (12-29 months) male and female mice of the C58, Balb/c, and C3H/HeN strains were compared for their capacity to become activated by various adjuvants in three assays. The results are as follows: (1) Chemiluminescence: Activation by phorbol myristic acetate and zymosan was increased two fold in macrophages from aging as compared to young mice, (2) Hexose

monophosphate shunt (HMPS): Activation by lipopolysaccharide (LPS) and polyadenylic-polyuridylic acid complexes (poly A·poly U), was readily achieved (1.5-2 fold) in macrophages from young mice, whereas it could not be achieved in cells from aging mice, and (3) Phagocytosis: Findings similar to HMPS were seen in this assay system with a stimulation index of 2-4 fold on macrophages of young mice and no activation of cells from aging mice. In contrast, macrophages from aging (12 months) C58 breeder females were not impaired in their ability to become activated in either the HMPS or phagocytosis assay. No significant differences between male and female mice were seen in any of the other tests. These results were similar qualitatively in all three strains, with the exception that HMPS activation occurred in both young and aging mice of the Balb/c strain. Thus macrophages from aging mice differ from young mice in their ability to become activated, and the source of these differences currently is being investigated.

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ACTIVATION OF ADENYLATE CYCLASE (AC) IN GUINEA PIG MACROPHAGE MEMBRANES IS REGULATED BY GUANINE NUCLEOTIDES. Margrith Verghese* and Ralph Snyderman. Howard Hughes Med. Inst., Duke Univ. Med. Ctr., Durham, N.C. 27710.

Macrophage functions such as chemotaxis, phagocytosis, secretion and cytotoxicity are influenced by cyclic nucleotide levels but the regulatory mechanisms involved are poorly defined. We therefore developed methods to study the direct activation of AC in guinea pig macrophage membranes. Such membranes are readily obtained from glycogen-induced peritoneal macrophages. Basal levels of cAMP production in the membranes were 1.5 $\pm$ 0.2 pM cAMP/min/mg protein. These levels could be raised to 87.8 $\pm$ 1.5 pM cAMP/min/mg protein by 10 mM NaF. In the absence of guanine nucleotides, hormones (e.g. isoproterenol, PG's) did not stimulate cAMP production. When the guanine nucleotides (GTP) or its nonhydrolyzable analog Gpp(NH)p were added a ca. 3 fold stimulation over basal levels was produced by hormones: in the presence of 0.1 mM GTP, membranes produced 56.3 $\pm$ 10.9 pM cAMP/min/mg protein while addition of 0.01 mM PGE_1 or 0.1 mM isoproterenol raised this to 158.7 $\pm$ 6.8 and 167.7 $\pm$ 9.6 pM cAMP/min/mg protein, respectively. Thus, hormone but not NaF induced stimulation of AC required guanine nucleotides. Hormone induced AC activation with GTP ceased after 15 min but with Gpp(NH)p activation continued for at least 40 min. This suggests that the macrophage membranes contain GTP-ase which hydrolyzes GTP. Enzyme activation by hormones was receptor specific since the effect of isoproterenol was blocked by propranolol and the potency series of the PG's corresponded to that observed for cAMP production in intact g.p. macrophages (i.e. $PGE_1 = PGE_2 > PGA_1 > PGF_{2\alpha}$). These methods now allow the study of the biochemical regulation of adenylate cyclase production in macrophage membranes.

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MACROPHAGE RECEPTORS FOR PHORBOL DIESTERS: COMPARABLE RECEPTORS IN NORMAL AND "ACTIVATED" CELLS DESPITE DIFFERENT CELLULAR RESPONSES. J. BRICE WEINBERG. VA and Duke Medical Centers. Durham, NC 27705

Mouse peritoneal macrophages respond to environmental stimuli in different ways depending on their state of differentiation. Macrophages from mice infected with BCG are known to produce more H_2O_2 in response to the phorbol diester phorbol myristate acetate (PMA) than do those from normal mice or mice injected with sterile inflammatory stimulants. Phorbol diesters mediate their pleiotypic effects on cells via specific membrane receptors. The purpose of this study was to determine if the different degress of responsiveness to phorbol diesters was related to differences in the cellular receptors for these ligands. Phorbol diester binding to macrophages was determined using tritium-labeled phorbol dibutyrate (3HPDBu) in a filtration binding assay in microtiter plates. The peritoneal cells were rid of erythrocytes and neutrophils by ficoll-hypaque centrifugation, and lymphocytes were removed from the adherent macrophages by repeated washes. As seen in the table, Scatchard analysis showed linear plots with all three types having specific, high affinity receptors for the 3HPDBu with comparable affinity (Kd) and number of binding sites/cell. However, they differed dramatically in their ability to produce H_2O_2 in response to PDBu. The binding was rapid and reversible. The ability of various phorbol diesters or analogues to induce H_2O_2 production by BCG macrophages correlated well with their known in vivo tumor promoting ability and their abilities to compete for 3HPDBu binding (PMA>PDBu>mezerein>>phorbol diacetate with phorbol inactive). These results suggest that the enhanced ability to produce H_2O_2 in response to phorbol diesters associated with in vivo differentiation is not caused by changes in the receptor for the ligand and that "post receptor" changes (e.g. coupling of the receptor with NADPH oxidase, or induction or activation of NADPH oxidase) account for the noted differences.

	Kd	sites/cell	max H_2O_2/10^6 cells/hour	ED50
Resident:	60nM	560,000	0	--
Peptone:	66nM	730,000	75	75nM
BCG:	91nM	720,000	120	54nM

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MOUSE MACROPHAGE PROCOAGULANT(S): EVIDENCE FOR VITAMIN K DEPENDENCE. J.W. SHANDS, JR. Dept. of Medicine, Univ. of Florida, College of Medicine, Gainesville, FL 32610.

Macrophages and monocytes of a variety of animals synthesize procoagulants (PCA) upon stimulation in vitro. These PCAs have been variously reported to be tissue factor or serine esterases which activate factor X or prothrombin. In these experiments mouse peritoneal macrophages elicited by Thioglycolate were incubated in serum free medium in vitro with endotoxin for 5 to 7 hrs. PCA activity was assayed in human plasma. Maximal PCA activity required the interaction of soluble supernatant factors from macrophages (control or stimulated) with lysates of stimulated cells. The resulting PCA activity could be sedimented at 100 KG. The generation of this PCA activity was inhibited by Actinomycin D (10 ug/ml), Tunicamycin (0.5 ug/ml) and by warfarin ($5X10^{-6}$ M). Warfarin inhibition could be reversed by the addition of Vitamin K_1 in excess. Analysis of the effect of metabolic inhibitors on the generation of supernatant factor(s) showed that the production of the supernatant factor(s) was inhibited by cycloheximide and warfarin but was not influenced by actinomycin D or by Tunicamycin. Comparisons of the activity of mouse brain "tissue factor" and macrophage PCA in normal and in deficient plasmas showed that the two could be distinguished. PCA was active in factor VII deficient human plasma. The data indicate that exudate macrophages produce vitamin K dependent clotting factors which can be activated by lysates of stimulated cells. The PCA of mouse exudate cells is composed of one or more of these factors which acts either at factor X or prothrombin.

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CHARACTERIZATION OF THE PROCOAGULANT ACTIVITY (PCA) INDUCED IN RABBIT KUPFFER CELLS (KC) BY BACTERIAL LIPOPOLYSACCHARIDE (LPS). **R. V. MAIER*, (Introduced by: E. Stan Lennard). Department of Surgery, University of Washington, Seattle, WA 98104.**

Circulating monocytes and non-hepatic macrophages have been shown to produce an inducible PCA (tissue thromboplastin) using various agents (including LPS). This PCA is postulated to contribute to the initiation of the disseminated intravascular coagulation (DIC) seen in clinical sepsis. Fibrin deposition associated physically with KC in the hepatic microcirculation is an early event observed after the intravenous injection of LPS. The ability of a homogeneous population of explanted rabbit KC to express a PCA was examined and the PCA characterized. Results represent the mean of three to six separate experiments run in triplicate. Addition in vitro of 10ng to 10µg of LPS from E. coli 0111:B4 stimulated a 10-20 fold increase in PCA within 8 hours as measured by a one-step thromboplastin assay. The PCA was >95% localized to the cellular lysate. In contrast to typical brain thromboplastin; however, KC PCA was markedly sensitive to inactivation at 56°C (<15% of control levels at 30 min.) and 1mM DFP inhibition (<10% of control levels). Additionally, coagulation assays using both the one-step thromboplastin assay and enzyme assays utilizing a Factor Xa specific substrate with normal and deficient human plasmas reveal an apparent direct activation of Factor X which is not dependent on the presence of Factor VII. Preincubation of the KC with Coumadin (20µg/ml) inhibits the production of PCA in response to LPS (<15% of untreated levels). Repletion with Vit. K1 reversed the blockade of PCA production. These studies demonstrate that LPS induces a PCA in KC that is functionally similar to Factor VII and dependent on Vit. K. A role for KC in the initiation of DIC in endotoxemia is also supported. **(Supported by NIH Grant GM 30272)**

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INHIBITION OF MURINE MACROPHAGE MIGRATION BY THE LEUKOCYTOSIS PROMOTING TOXIN (LPT) OF BORDETELLA PERTUSSIS. B.D. MEADE*, P.D. KIND*, C.R. MANCLARK*.(Introduced by: S.K.Ackerman). Bureau of Biologics Bethesda, MD 20205 and George Washington University, Washington, D.C. 20037.

LPT is a protein toxin which may have a role in the pathogenesis of pertussis. Since macrophages have an important role in controlling respiratory infection, the effects of LPT on macrophage function were examined using murine peritoneal macrophages. LPT was purified to homogeneity as assessed electrophoretically, and shown by limulus assay to contain less than 0.01% endotoxin. Injection (i.v.) of NIH mice with 80ng or 400ng LPT one hour before intraperitoneal injection of thioglycollate broth decreased by 37% or 76%, respectively, the number of macrophages recoverable by peritoneal lavage 3 days later. LPT(80ng) injected i.v. increased the peripheral leukocyte count 3 days later by 15,000 cells/mm^3, and 400ng increased the count by 50,000/mm^3. Thus doses of LPT which alter macrophage migration to the site

of inflammation are similar to those which produce leukocytosis. Migration in vitro was assessed by counting the cells migrating through membrane filters in response to the attractant, endotoxin activated mouse serum. Less than 1ng/ml LPT decreased by more than 50% the migration of C3H/HeN and C3H/HeJ resident peritoneal macrophages. At doses 2-5 fold higher, the migration of the murine macrophage-like cell line, RAW264, was also inhibited. LPT alone is not chemotactic. The inhibitory effects of LPT on in vitro migration were abolished by heating (80°C, 30 min.) or by mixing with specific antibody. Doses up to 50ng/ml do not decrease the trypan blue viability of macrophages. The data suggest a possible role for LPT in pathogenesis by inhibiting migration of macrophages to the site of infection.

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NEUTRALIZATION OF HOST DEFENSE MECHANISMS BY GBS CAROTENOID PIGMENT. R.A. NEMERGUT,* K. MERRITT Orthopaedic Research, TB 139, University of California, Davis, CA 95616.

A red-orange carotenoid pigment is produced by most human strains of group B streptococci (GBS) when grown anaerobically. The importance of this pigment in the pathogenesis of GBS disease may be as an electron sink, protecting the organisms from the lethal effects of superoxide or other oxygen metabolites. To test this hypothesis, pigmented and nonpigmented organisms were incubated in the presence of superoxide generated by the photoactivation of riboflavin. Unneutralized superoxide was detected by the reduction of cytochrome C. The amount of superoxide present when incubated with the nonpigmented organisms was at least twice that found with the pigmented cultures. The second approach was to test the ability of superoxide and hydrogen peroxide to kill the organisms. This was measured by incubating several strains of pigmented and nonpigmented organisms in the presence of photoactivated riboflavin or hydrogen peroxide, sampling at 30 minute intervals, and plating for colony counts. The nonpigmented strains were rapidly killed while the pigmented organisms declined slowly after initial growth. Results of these studies indicate the protective role of the carotenoid pigment. Pigmented organisms have an increased ability to neutralize superoxide and survive. Thus the pigment is important in neutralization of host defense mechanisms.

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RADIOIODINATED β-GLUCURONIDASE ENTERS THE PARASITOPHOROUS VACUOLES OF MACROPHAGES INFECTED WITH LEISHMANIA MEX.AMAZONENSIS. V.L. Shepherd*, P.D. Stahl, D. Biegel*, G. Topper*, M. Rabinovitch. Dept. of Physiol. & Biophys., Washington U. Sch. of Med., St. Louis, MO 63110 and Dept. of Cell Biol., N.Y.U. Sch. of Med., New York, NY 10016.

Leishmania are nearly obligatory parasites of macrophages. Non-flagellated amastigotes lodge and multiply within parasitophorous vacuoles (p.v.). Studies with electron-opaque tracers emphasized the phagolysosomal nature of p.v. (Berman et al., J. Protozool. 28:239,1981). We show that β-glucuronidase (β-gluc), a lysosomal enzyme pinocytosed by macrophages via a specific mannose receptor (Stahl et al., Cell 19:207, 1980), enters p.v. as judged by light microscopic radioautography. Bone marrow-derived mouse phagocytes were infected with amastigotes of L.mex.am., which characteristically form very large p.v. Cultures were kept at 34°C and incubated with ^{125}I-labeled β-gluc. Receptor-mediated uptake of ^{125}I-β-gluc was identical in control and infected cultures, and increased with time (1-24 h). By radioautography, grains were associated with p.v., with the surface of small basophilic cells and with the cytoplasm of uninfected cells. There were few grains over the cytoplasm of infected macrophages. Detectable vacuolar uptake began at 5 h and increased at 24 and 40 h, when 50% or more of the vacuoles were labeled. Incubation with mannan lowered uptake. Some p.v. were labeled in macrophages incubated 20 h with the ligand followed by 24 h in ligand-free medium. A few labeled vacuoles were found in cells given the ligand prior to infection. The specific uptake of β-gluc confirms the fusion of lysosomes with p.v. and suggests that the enzyme could be used as a drug carrier able to reach intraphagosomal parasites of macrophages. Supported by grants 1541A (Cystic Fibrosis Fdn.) to VLS; CA 12858 and GM 21096 to PDS; and AI-10969 to MR.

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OXYGEN-INDEPENDENT ACTIVITY AGAINST INTRACELLULAR PATHOGENS: TOXOPLASMA GONDII AND CHLAMYDIA PSITTACI. G.I. BYRNE*, C.D. ROTHERMEL, and H.W. MURRAY. Cornell University Medical College, New York, NY 10021.

We have previously demonstrated that macrophage (MAC) oxidative activity correlated closely with the ability to kill or inhibit the replication of intracellular T. gondii. In addition, techniques that diminished the oxidative capacity of activated MACS (phorbol myristate acetate (PMA) pretreatment, deprivation of glucose, exogenous catalase treatment) reversed antitoxoplasma activity. In contrast, these manipulations have no effect on the ability of lymphokine (LK)-activated MACS to inhibit C. psittaci replication.

We now report that mitogen-induced LK's stimulated mouse fibroblasts to inhibit T. gondii and C. psittaci multiplication in the absence of significant oxidative activity in assays that measured chemiluminescence, H_2O_2 release, and nitroblue tetrazolium (NBT) reduction. In addition, although heating LK to 56° for 60 min ablated the component that enhanced MAC chemiluminescence, it did not affect the capacity of LK to activate MACS (and fibroblasts) to inhibit T. gondii and C. psittaci replication. The capacity of LK-stimulated fibroblasts to restrict the growth of these organisms was also not affected by PMA pretreatment, glucose deprivation, or the presence of catalase. Fractionation of LK by gel filtration yielded two distinct components; one enchanced MAC chemiluminescence, the other stimlulated chlamydiastatic activity.

We conclude that oxygen-independent mechanisms may be of more importance than previously thought in the ability of MACS and "non-professional" phagocytic cells to inhibit growth of intracellular pathogens.

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KILLING OF LISTERIA MONOCYTOGENES BY INFLAMMATORY NEUTROPHILS AND MONONUCLEAR PHAGOCYTES OBTAINED FROM IMMUNE AND NON-IMMUNE MICE. C.J. CZUPRYNSKI, P. HENSON AND P.A. CAMPBELL. National Jewish Hospital and Research Center, Denver, CO 80206

Using a suspension bactericidal assay, we have shown that inflammatory peritoneal macrophages and neutrophils from mice can kill 90% or more of the total Listeria during a 2 hr incubation period. The expression of in vitro listericidal activity by inflammatory peritoneal phagocytes followed a consistent time course. Peritoneal neutrophils obtained early during the inflammatory process (4 hours after elicitation) were listericidal, whereas neutrophils obtained 24 hours after elicitation had a diminished ability to kill Listeria. In contrast to resident peritoneal macrophages or macrophages obtained 4 hours after elicitation, which were unable to kill Listeria, macrophages obtained later during inflammation (maximal activity at 48 hours after elicitation) were able to kill Listeria in vitro. Inflammatory peritoneal neutrophils and macrophages exhibited a similar time course in their expression of bactericidal activity against either L. monocytogenes or E. coli. Although intraperitoneal immunization or intravenous hyperimmunization markedly enhanced resistance of mice to Listeria in vivo, immunization did not affect inflammatory peritoneal phagocyte listericidal activity in vitro. However, in response to intraperitoneal injection of proteose-peptone or dead Listeria, immunized mice mobilized greater numbers of neutrophils and mononuclear phagocytes into the inflamed peritoneum than did non-immune mice. These data suggest that, rather than systemic activation of mononuclear phagocytes, increased recruitment of neutrophils and mononuclear phagocytes into sites of infection may be of prime importance in resistance to listeriosis.

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THE IDENTIFICATION OF LYMPHOKINES PRODUCED BY A HUMAN T CELL LINE WHICH INHIBIT OR ENHANCE THE INTRAMACROPHAGE REPLICATION OF MYCOBACTERIUM TUBERCULOSIS, OR INDUCE THE FORMATION OF MULTINUCLEATE GIANT CELLS, IN HUMAN MACROPHAGE CULTURES. G.S. DOUVAS, D. ANDERSON, A.J. CROWLE. Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Eleanor Roosevelt Institute for Cancer Research, Denver, CO 80262.

The human T cell line HSB-2, when stimulated with phytohemagglutinin and phorbol myristate acetate, produces lymphokines which (1) induce human macrophages to inhibit the replication of infecting tubercle bacilli (GIF), (2) stimulate the intramacrophage replication of tubercle bacilli (GEF), and (3) induce the formation of multinucleate giant cells in macrophage cultures (GCF). To demonstrate GIF activity, human monocytes are cultured for seven days in RPMI with one percent unheated autologous serum and then infected with tubercle bacilli. HSB-2 supernates are then added to the infected macrophage cultures. Five days later the number of acid-fast bacilli

per infected macrophage is determined. GEF activity is demonstrated on macrophages grown, infected and treated with HSB-2 as above, except that the cells are cultured in Neuman-Tytell medium. GEF can also be shown in RPMI, but it must first be separated from GIF in whole HSB-2 supernates by gel filtration. GCF activity is measured by adding HSB-2 supernate to seven-day macrophage cultures grown in Neuman-Tytell, and then counting the number of multinucleate giant cells 48 hours later. HSB-2 supernates do not affect bacillary growth in 7H9 medium in the absence of macrophages, as measured by turbidimetry. Our results indicate that macrophages respond differently to lymphokine containing supernates, depending on the conditions used for culture. (Supported by U.S. National Research Service Institutional Award No. T32A107035-06.)

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MODULATION OF THE EEL IMMUNE SYSTEM BY ETE. L.J. MCCUMBER, M.M. SIGEL, E. HUGGINS, J. DAVIS AND S. HAYASAKA, Department of Microbiology & Immunology, USC School of Medicine, Columbia, SC 29208 and Department of Microbiology, Clemson University, Clemson, SC 29631.

Ete, an extract derived from the marine tunicate Ecteinascidia turbinata, was found to alter several parameters of the defense system of the American eel Anguilla rostrata. Exposure of eel peripheral blood leukocytes to Ete in vitro caused an enhancement of binding and phagocytosis of ^{51}Cr sheep red blood cells. Intravenous injection of Ete was found to enhance the immune system of the eel as measured by three criteria: 1) production of antibody to bacteria, 2) phagocytic activity of leukocytes, and 3) granulocytosis. Ete increased resistance to infection with Aeromonas hydrophila, a bacterium which normally causes furunculosis and extensive mortality in eels. The effect was apparently the result of potentiated phagocytic activity and increased release of granulocytes into circulation rather than augmented antibody responses.

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REACTIVITY OF ANTIBODY TO ASIALO-GM1 WITH ELICITED AND ACTIVATED MACROPHAGES. R.H. Wiltrout*, A.Santoni*, E.S.Peterson*, R.B.Herberman and H.T.Holden, Biological Research and Therapy Branch, NCI-FCRF, Build. 560/Rm. 31-93, Frederick, MD 21701.

Antiserum to the neutral glycolipid asialo-GM1 (asGM1) abrogates the in vitro and in vivo cytolytic activity of natural killer (NK) cells and enhances the formation of artifically induced B16 melanoma metastases in the lung and liver of C57BL/6 mice. The studies we will report have been designed to examine the reactivity of anti-asGM1 serum with elicited and activated macrophages in order to further delineate the relative contributions of NK cells and macrophages against tumor growth and metastases in vivo. Treatment with anti-asGM1 plus complement of peptone-elicited macrophages in vitro before they were exposed to activating agents in vitro resulted in only minimal reduction of the levels of tumor cell cytolysis. However, in vitro treatment of thioglycollate-elicited macrophages or macrophages activated in vivo with MVE-2 or poly I:C/L:C resulted in a marked decline in cell viability and decreased levels of cytotoxicity of the activated cells. In addition, macrophages from animals that were treated with one of several activating agents and a dose of anti-asGM1 that would totally ablate NK activity, had cytolytic activity that was substantially reduced but not totally abrogated. Studies are currently underway to determine the distribution of asialo-GM1 on macrophage subpopulations to evaluate whether this may be a marker of different stages of macrophage activation and/or differentiation and whether this may be useful in delineating the functional role of macrophages in anti-tumor host responses.

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3':5'-CYCLIC ADENOSINE MONOPHOSPHATE REGULATES HAMSTER NATURAL KILLERS AT TWO DIFFERENT LEVELS. S. FAN, W. TOMPKINS. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL 61801.

We examined the possible roles 3':5'-cyclic adenosine monophosphate (cAMP) may play in regulating natural killers (NK) in the Syrian harster. To detect cytotoxic

effectors in either spleen mononuclear cells or in vitro generated effector populations, we used a previously described ^{51}Cr release assay with baby hamster kidney cells infected with herpes simplex virus (BHK_H) as targets. Dibutyryl cAMP (dbcAMP) or theophylline incorporated into cytotoxicity assay mixtures reduced NK cytotoxicity without affecting the amount of ^{51}Cr spontaneously released from the targets. Similarly, effectors treated with cholera toxin (CT) before assay exhibited lower cytotoxicity than untreated controls. This suggests that at the effector level, high intracellular cAMP concentrations suppress cytotoxicity. Chapes et al. demonstrated that macrophages from hamsters previously immunized with vaccinia virus (IMP) stimulate mononuclear bone marrow cells (BMC) to mature into NK. This maturation response did not occur in IMP-BMC cultures that also contained theophylline or dbcAMP. IMP treated ≥ 4 hr with dbcAMP or CT were less capable of promoting BMC maturation to NK than untreated controls, whereas BMC treated with dbcAMP were not altered in their maturation to NK. Our data suggest that depending on the cell type affected, intracellular cAMP levels may regulate not only NK's ability to cause cellular injury, but also their generation from BMC.

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DISSOCIATION OF NK ACTIVITY AND RESISTANCE TO URETHANE-INDUCED LUNG ADENOMAS. S. LEMIEUX, E. SKAMENE. Institut Armand-Frappier, Université du Québec, Laval, Québec H7V 1B7 and Montreal General Hospital, Montreal, Québec H3G 1A4.

The in vitro NK activity of splenocytes against YAC-1 target cells and the in vivo resistance to urethane-induced lung adenomas are two traits which are genetically regulated in the mouse. There is a correlation between low NK activity and susceptibility to urethane oncogenesis in some inbred strains (e.g. A) and between high NK cell activity and resistance to urethane oncogenesis in other strains (e.g. B10.A). We have analyzed whether these two putatively linked phenotypes would remain associated among segregating backcross progeny derived from those 2 parental strains. The design of the experiments was such that NK cell activity (both pre- and post-urethane treatment) and number of lung adenomas induced by urethane could be determined, successively, in the same individual animal. We found that the NK activity of (B10.A x A)F_1 hybrid mice was as high as that (B10.A) parents, and that it could be equally well suppressed by urethane. In contrast, while B10.A mice were fully resistant to urethane-induced oncogenesis, the (B10.A x A)F_1 hybrids were semi-susceptible. Furthermore, a clear-cut dissociation between the level of NK activity and the number of urethane-induced lung tumors was observed among the individual progeny of [(B10.A x A)F_1 x A] backcross. These results argue against the role of NK cells in resistance to urethane-induced tumors and show that the association of low NK activity and tumor susceptibility, or high NK activity and tumor resistance as found in some inbred strains, is fortuitous. (Supported by grants from MRC 6431 and USPHS AI 18693).

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Role of NK cells in the inhibition of tumor growth by interferon. Timothy L. Ratliff, Dov Kadmon*, Dennis M. Oakley*, Amos Shapiro* and William J. Catalona*. Washington University School of Medicine at The Jewish Hospital, Department of Surgery (Urology), St. Louis, MO 63110.

We studied the mechanism by which interferon [both murine L cell interferon (IFNα,β) and gamma interferon (IFNγ)] inhibits the growth of the transplantable N-[4-(5-nitro-2-furyl)-2-thizolyl]-formide (FANFT)-induced bladder tumor, MBT-2. C3H/HeJ female mice were injected intramuscularly in the hind leg with 10^4 viable MBT-2 cells, and therapy was initiated on the same day by injecting 500 units IFNγ and 1000 units IFNα,β into the tumor-bearing leg. Therapy was continued for 7 consecutive days and at 3 day intervals therafter for 4 injections. Controls included injection of mock interferon and phosphate buffered saline (PBS). Mice treated identically were sacrificed at various times during treatment to monitor NK activity. The results show a significant inhibition of tumor outgrowth by both IFNα,β and IFNγ as compared to controls. Twenty-four days after tumor injection 80% of the PBS- and 75% of mock-treated mice had palpable tumors while only 40% of IFNα,β and 15% of IFNγ-treated mice had tumors after 90 days. Immunological studies performed throughout the therapy protocol revealed no augmentation of NK activity in interferon-treated mice. In separate in vitro clonogenic assays, we observed a direct inhibition of MBT-2 colony formation by both IFNα,β and IFNγ. These results suggest that the anti-tumor activity observed in our model is a localized phenomena which may

be attributable, at least in part, to direct inhibition of tumor cell division and not from systemic augmentation of NK activity. (Supported by CA 28860 from NCI through NBCP).

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EFFECTS OF FASTING ON IMMUNITY IN HUMANS: DISSOCIATION OF CELLULAR FUNCTION. E.J. WING, A.W. WINKELSTEIN. Montefiore Hospital, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Little data exist on the effect of acute nutritional deprivation on immune function in man. As an approach to this problem, we studied 15 obese human subjects who were fasted for 14 days under metabolic ward conditions. A range of immunological parameters was assessed before and at the end of the fast including peripheral blood (PB) monocyte bactericidal activity against S.aureus; PB natural killer (NK) cell cytolysis of K562 cells; total PB lymphocyte blastogenic responses to mitogens; and delayed hypersensitivity (DH) responses to antigen. Fasting increased monocyte bactericidal activity in 12 of 14 subjects ($P<.05$); similarly, NK cell cytolytic activity in-increased in 11 of 13 subjects tested ($P<.02$). The increase in specific cytotoxicity averaged 24%. By contrast, lymphocyte blastogenic responses to PHA were decreased by 19% ($P<.01$). Lymphocyte responses to pokeweed mitogen were not significantly decreased. DH responses to either SKSD or candida were present in all subjects before and at the end of fasting. The total number of PB lymphocytes, T cells, and B cells were not changed by starvation. These results indicate that fasting has differential influences on immune function rather than a uniformly deleterious effect. Of potential import, this nutritional abnormality appears to actually enhance certain effector functions of the host defense system.

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EFFECT OF Ca^{2+} ION INFLUX AND CALMODULIN ACTIVITY ON MACROPHAGE CYTOTOXICITY. HUE DUY, W. TOMPKINS. Department of Veterinary Pathobiology, University of Illinois, Urbana, Illinois 61801.

To examine the effects of Ca^{2+} influx and calmodulin activity on macrophage cytotoxicity, we used ^{51}Cr labeled BHK (baby hamster kidney) cells infected with HSV (herpes simplex virus) as targets and Syrian golden hamster adhered macrophages (MØ) as effectors in a 16 hour cytotoxicity assay. Addition of A23187 ionophore to the assay enhanced MØ cytotoxicity as compared to untreated cells. At the most effective concentration of 5 ug/ml, A23187 significantly increased lysis but had no effect on ^{51}Cr release. This effect was not seen when the effector cells were pretreated with A23187. Chlopromazine (10μM) and trifluroperazine (TFP 10 μM) abgrogated the effects of A23187 on MØ cytotoxicity when added to the assay, suggesting that calmodulin is an integral component in the ionophore induced MØ cytotoxicity. Theophylline (1 mM) and dibutyryl cAMP, (1 mM), reduced the ionophore induced MØ cytotoxicity when added with A23187 to the assay. indicating that this cytotoxic activity is related to the increased degradation of cAMP. Addition of cholera toxin (CT, 5 μg/ml) to the assay suppressed the A23187 induced MØ response. Pretreatment of MØ with CT (5 μg/ml) gave a similar effect, indicating that the ionophore enhanced cytotoxicity at the effector level. Our data suggests that MØ cytotoxicity depends on the Ca^{2+} influx across the MØ membrane and these effects are regulated by calmodulin and may be expressed through the cyclic nucleotide pathway.

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REQUIREMENT OF GLYCOSYLATED PROTEIN FOR HUMAN NK EFFECTOR CELL RECOGNITION. ANN HINSON*, EDWIN W. ADES. Lilly Research Laboratories, Indianapolis, Indiana 46220

One possible explanation for effector-target cell interaction involving human natural killing (NK) may be that target cells are recognized by receptors on natural killer cells specific for cell surface carbohydrates. To test whether carbohydrate structures on target cells are necessary for recognition, we have treated an NK susceptible tumor target cell line (K562) with tunicamycin (a protein glycosylation inhibitor) and then examined the treated cells for their susceptibility to lysis by human NK cells. Our experiments show that treatment of K562 cells with doses of tunicamycin from 2 µg/ml to 20 µg/ml caused inhibition of NK activity in a

concentration-dependent manner. This inhibition was observed to decrease over a four day period demonstrating recovery of susceptibility to lysis by the tumor target cell. Tunicamycin treatment had no effect on cell viability. We found that tunicamycin has no effect on protein synthesis of K562 cells, wheras glycoprotein synthesis was significantly inhibited. In addition, treatment of effector cells with tunicamycin caused inhibition of lysis. In previous work we have shown that certain monosaccharides can inhibit NK activity, and that treatment of effectors with mixed glycosidases completely inhibits NK activity. Taken together, these data strongly suggest the need for glycosylated protein on the target cell surface as well as on the effector cell in order to obtain the recognition and binding necessary for lysis.

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IMMUNOLOGIC AND VIRAL PROTECTIVE PROPERTIES OF BORDETELLA PERTUSSIS ACELLULAR FRACTIONS 15A-1B AND 15A-108A (CONNAUGHT LABORATORIES, SWIFTWATER, PA). ROBERT S. STINSON, J. DENNIS LEE*, LISA WILLIAMSON*, and ALVIN WINTERS*. Department of Microbiology, University of Alabama, University, AL 35486.

Since the introduction of the whole cell Bordetella pertussis vaccine (BPV), the incidence of whooping cough has declined dramatically in the United States and most of the Western World. Numerous adverse side effects of BPV have been documented. BPV and its acellular components are currently being analyzed in terms of lessening the severity of vaccine-related complications. An endotoxin-free fraction (15A-108A) did not induce the splenomegaly associated with both BPV and 15A-1B (high salt, neutral pH) with spleen weights (mg) of 104.7 $\pm$ 15.7, 314.6 $\pm$ 97.2, and 171.9 $\pm$ 43.1 for 15A-108A, BPV, and 15A-1B, respectively. In addition 15A-108A did not induce the delayed type hypersensitivity associated with both BPV and 15A-1B. Immunomodulation by BPV in mice is well established but little information is available concerning BPV effects on viral infections. We have shown previously that administration of BPV 7 days prior to a lethal dose of mouse adenovirus resulted in complete protection for approximately 5 weeks. 15A-1B protected approximately 81% of BDF_1 mice and 15A-108A exhibited 80% protection against lethal challenge. BPV exhibited 100% protection and all control (unimmunized) mice died within 10 days post-challenge. Preliminary studies indicate a peak in immune interferon production prior to day 4 post-vaccine administration. Studies are underway to determine the mechanism of protection and to assess the possible role of interferon in this transient protection against adenovirus infections.

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THE INDUCTION OF COMPLEMENT RECEPTOR 3 (CR3) ON MACROPHAGES BY AN IN VIVO-DERIVED DIFFERENTIATION ACTIVITY. S.-E. YEN*, W.S. WALKER. Division of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38101.

Peritoneal resident macrophages (PRMØ) from normal mice bear CR1 and CR3 (bind complement components C3b and iC3b, respectively). By contrast, most MØ in elicited peritoneal exudates (PEMØ) bear CR1 but lack CR3, as do MØ derived from progenitors in bone marrow (BM) and peripheral blood (PB). The unique CR phenotype on normal PRMØ suggests that CR3 might be a useful marker in studies on the origin(s) and development of various MØ populations. We report here that cell-free lavage fluids from the peritoneal cavities of normal mice contain an activity capable of inducing CR3 on a portion of PEMØ and on MØ in colonies derived from progenitors in the PE population. This MØ differentiation activity (MDA) is stable at 56°C for 30 minutes but destroyed at 70°C and is also trypsin-sensitive. Furthermore, MDA is not CR3, and "converted" CR3(+) MØ regenerate their CR3 in the absence of MDA after removal of the receptors with trypsin. Lastly, MDA fails to influence the CR3 phenotypes of BM- and PB-derived MØ. These results show that MDA affects a stable CR3 phenotype in a subpopulation of PEMØ and documents the effect of local microenvironment on the development of a particular population of MØ. (Supported by NIH Grant CA16652, NCI Cancer Center Support (CORE) Grant CA21775 and ALSAC)

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T CELL HYBRIDOMA PRODUCTION OF LYMPHOKINE ACTIVITY THAT INTERFERES WITH PGE_2-MEDIATED NEGATIVE REGULATION OF MACROPHAGE ACTIVATION. S.W. RUSSELL and J.L. PACE. [2]Department of Comparative and Experimental Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610.

The hybridoma, which was produced by fusion of alloantigen activated mouse T lymphocytes and BW 5147 lymphoma cells (J. Exp. Med., 152, 1980:956), was a gift from Drs. R. Schreiber, D. Katz, and A. Altman (Scripps Clinic, La Jolla, CA). Concanavalin A stimulation (5 ug/ml) of a subclone in serum free Dulbecco's medium caused the production of lymphokine activity that interfered with the ability of PGE_2 (10^{-8}M) to shut off bacterial lipopolysaccharide (Escherichia coli, 0111:B4; 100 ng/ml)-mediated activation of mouse (C3H/HeN) resident peritoneal macrophages for tumor cell killing (^{51}Cr labeled P815 mastocytoma cells). This activity was functionally similar to that which is produced by Con A stimulation of mouse spleen cells (J. Immunol., 127, 1981:121). Supernates from cultures of the cloned T cell hybridoma also contained antiviral activity attributable to interferon gamma. Rabbit polyclonal antibody against interferon gamma, a gift from Dr. H. Johnson (University of Texas, Galveston), neutralized the antiviral activity, as well as the one that interfered with PGE_2-mediated negative regulation of macrophage activation, suggesting that the latter activity was attributable to interferon gamma. Supported by research grant CA 31199 from the National Cancer Institute and Biomedical Research Support Grant RR05788-05.

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THE ROLE OF MACROPHAGES AND FUNCTIONAL T-CELL SUBSETS IN THE LEUKOCYTE (MACROPHAGE) ADHERENCE INHIBITION (LAI) MEASURE OF CELLULAR IMMUNITY TO MURINE SARCOMA VIRUS (MSV) - INDUCED TUMORS. Katherine Sarlo* and Richard F. Mortensen, Dept. of Microbiol., Ohio State Univ., Columbus, Ohio 43210.

Sensitized spleen T-lymphocytes from tumor-bearing animals elaborate a variety of lymphokines including LAI factor (LAIF), in response to membrane tumor-associated antigens. We have previously reported that the decreased affinity of macrophages for plastic surfaces is mediated by LAIF produced by sensitized T-cells from mice with regressing MSV-induced tumors. MSV-immune spleen cells (ISC) were assessed for the functional T-cell subpopulations involved in LAIF production by the indirect LAI procedure using the murine leukemia-virus induced cell line RBL-5 as an antigen source. Lyt-2/3 positive cells (cytotoxic/suppressor) produce LAIF in the absence of (adherent) macrophages, whereas Lyt-1 positive cells (helper) elaborate LAIF only in the presence of a small percentage of macrophages. Addition of increasing numbers of either normal or ISC macrophages, as well as their supernatants, nonspecifically suppressed the LAI response of both T-cell subsets. Tumor infiltrating macrophages inhibited the LAI response of Lyt-2/3 cells. A macrophage derived factor suppresses the LAI response of unfractionated T-cells and Lyt-2/3 cells. The results show that the elaboration of LAIF is not confined to a specific T-cell function subpopulation and is greatly influenced by spleen and tumor infiltrating macrophages. (Supported by Am. Cancer Society grant 16T).

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PURIFICATION OF MULTIPLE HUMAN MACROPHAGE ACTIVATION FACTORS (MAF). MILDRED C. McDANIEL. Quillen-Dishner College of Medicine, East Tennessee State University, Department of Biochemistry, Johnson City, TN 37614-0002

The assay used for the characterization and purification of human MAF is a 24 hour bioassay testing the cytotoxicity of mouse macrophages toward a syngeneic tumor cell line. MAF has multiple active species with molecular weights of 70,000, 25-30,000 and less than 10,000. These active molecules are produced both by the Namalwa lymphoma cell line as a constitutive product and by Concanavalin A stimulated human peripheral blood lymphocytes. In time studies on the production of MAF the smallest species (less than 10,000) is produced first. Purification procedures have been developed to solve the problem of the hydrophobicity of MAF and the adherence of MAF to serum proteins. Using 8M urea as a denaturating agent a DEAE column is used to remove 50% of the serum proteins. The chromatography on a Con A-Sepharose column equilibrated with 8M urea removes the remaining serum proteins. Final purification uses reverse phase high performance liquid chromatography. (Supported by NSF Grant #PCM-8007869).

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INFILTRATION OF HOST DEFENSE CELLS IN TUMORS. C.C. STEWART, S.J. STEWART AND A.P. STEVENSON. Los Alamos National Laboratory, Experimental Pathology, Los Alamos, NM 87545

Progressing tumors not only contain tumor cells but also normal host cells. Indeed, in many instances there may be more normal cells than tumor cells. These include not only endothelial cells and fibroblasts but also lymphocytes and macrophages. We are using collagenase in saline G to improve the yield of the disperse tumor. Using this method and the EMT6 or RIF tumors we improved yields of viable cells 5-10 fold. The presence of BSA apparently protects the cells from trypsin activity while the collagenase effectively breaks down the connective tissue. In order to evaluate lymphocyte and macrophage infiltration, MAC I was used to label macrophages, Lyt-1 was used to label T-cells and an anti-mu antibody was used to label B-cells. As a proof of principle, peritoneal exudate cells, spleen cells and cultured EMT6 cells were mixed together in known proportions, stained with the various reagents and analyzed by flow cytmetry. The results showed that each population could be resolved. Following these studies, the appropriate staining conditions were then applied to progressing tumors. Results of our studies quantifying lymphocyte and macrophage infiltration as a function of time will be presented. This work was performed under the auspices of the U.S. Department of Energy at the National Flow Cytometry Resource and was supported by Grant #CA33593 awarded by NCI.

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FIBRONECTIN LEVELS DURING GROWTH OF THE LEWIS LUNG TUMOR AS RELATED TO MACROPHAGE FUNCTION. R. Megirian, F.A. Blumenstock, J.A. Bennett* and T.M. Saba. Departments of Physiology and Surgery, Albany Medical College, Albany, NY 12208.

Macrophage phagocytic activity in relationship to immunoreactive and bioassayable plasma fibronectin was examined for 22 days after implant of the Lewis lung carcinoma (1×10^6) into the right front foot pad of C57BL/6 female mice. RES function was determined by blood clearance and localization of ^{131}I gelatinized RE test lipid emulsion. Plasma samples were evaluated for opsonic activity utilizing a mouse peritoneal macrophage monolayer assay and immunoreactive fibronectin was determined by electroimmunoassay. RES stimulation was observed over a 9-11 day period after tumor implant and was primarily associated with hepatic hyperphagocytic activity and reduced splenic and pulmonary uptake. While hepatomegaly developed in a later time period after tumor implant, enlargement of the liver did not exist over the 9-11 day period of hepatic RES stimulation. In contrast, plasma opsonic activity paralleled the changes in RES function with a pattern of elevation followed by decline. Immunoreactive fibronectin increased in parallel with elevated plasma opsonic activity but then remained elevated with a decline in bioassayable opsonic activity. Thus, enhancement of hepatic macrophage function early after tumor challenge appears primarily humorally mediated (opsonic activity). Sustained elevation in immunoreactive fibronectin at a time when plasma opsonic activity as mediated by this molecule begins to decline may reflect proteolytic fragmentation of fibronectin and/or release of antigenically related but non-opsonically active protein. (Supported by Institutional American Cancer Society Grant and AI-17635)

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THE EFFECT OF DIETHYLSTILBESTROL (DES) ON HOST RESISTANCE AND TUMOR SUSCEPTIBILITY IN MICE. J. BRADOF*, R. FUGMANN, C. ARANYI*, P. BARBERA*, J. FENTERS*. IIT Research Institute, Life Sciences Division, Chicago, IL 60616.

As part of a program to develop and validate methodology to measure chemically-induced immunotoxicity, the effect of DES on resistance of adult B6C3F1 female mice to various microorganisms and to challenge with syngeneic tumor cells was evaluated. The mice received s.c. injections of 50 µl of corn oil or oil containing the equivalent of 0.2, 1 and 4 mg/kg/day of DES for 14 days. Three days later they were challenged with *Listeria monocytogenes*, *Streptococcus* sp., influenza virus, herpes virus, *Trichinella spiralis* or B16-F10 tumor cells. Host resistance parameters were mortality for the bacterial and viral systems, expulsion of adult parasites from the gut for *T. spiralis* and lung weights for the B16-F10 tumor cell model. Host resistance to *L. monocytogenes*, herpes virus and *T. spiralis* was significantly decreased following DES exposure. Resistance to *Streptococcus* sp. was decreased, but not at a statistically significant level following these doses of DES. However, a dose of DES of

8 mg/kg/day resulted in a highly significant decrease in resistance to the organism. Resistance to influenza virus was unaffected by the DES. In contrast to the above, host resistance to i.v.-administered B16-F10 tumor cells was significantly increased as a consequence of DES exposure. These model systems for measuring alterations in host resistance have been indicated to hold potential for the routine screening of chemical and environmental agents for their possible immunomodulation. This includes not only adverse, but also stimulatory effects on the immune system of the host. (Supported by NIEHS Contract NO1-ES-1-5000).

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MODIFICATION OF A LYMPHOID CELL SUBSET IN PATIENTS WITH BREAST CANCER. M.F. LAVIA, E. CILLARI, G. DI GESU, S. PALMERI, D. LIO, A. SALERNO, P. LI VOTI Medical University of South Carolina Charleston, SC 29425 and University of Palermo, Italy

Lymphocyte populations which bear Fc receptors for IgG (FcγR) have been studied extensively with several techniques to assess their status in human pathologic conditions. The immunologic roles of these cells are quite important as they appear to contain the majority of T lymphocytes with suppressor activity. We have studied $Fc\gamma R^+$ lymphocytes by a new method employing E rosetting techniques in conjunction with a rosetting technique with chicken erythrocytes sensitized with rabbit anti-chicken erythrocyte IgG. In this fashion we have defined T lymphocytes and identified $Fc\gamma R^-$ lymphocytes in one simple step. By using this technique we have examined a group of women with nonmetastasized mammary carcinoma at different stages during their disease and compared them with a group of normal, age matched subjects. Our results indicate that $Fc\gamma R^-$ T lymphocytes detected by our mixed rosette technique are significantly reduced in mammary carcinoma patients. Thus we conclude that some changes occur in these patients which reduce a population of T lymphocytes which may be directly involved in a regulatory function of immunological responses.

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ANTIGEN-INDUCED PROLIFERATION OF THIOGLYCOLATE-ELICITED MOUSE PERITONEAL MACROPHAGES IN VITRO UNDER NORMAL CULTURAL CONDITIONS. P. L. Glover*, E. H. Perkins. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

Resident mouse peritoneal macrophages rarely synthesize DNA and divide in vitro. They can only be stimulated to proliferate minimally in the presence of cell-free inflammatory exudates or highly specialized conditioned medium. In contrast, macrophages elicited by the i.p. injection of thioglycolate are metabolically and functionally more active by a variety of criteria, and in the presence of macrophage growth factor proliferate extensively. We now report that adherence purified thioglycolate macrophages under normal cultural conditions (RPMI-1640 supplemented with 5% human serum) proliferate and this proliferation is dramatically enhanced in the presence of antigen (DNP-BGG). Evidence that this is ^{3}H-thymidine incorporation by antigen-stimulated macrophages and not contaminating lymphoid cells is as follows: 1) These cultures do not respond to PHA, Con A and LPS, 2) Column-separated splenic T cells in the presence of antigen and irradiated macrophages do not incorporate ^{3}H-thymidine, 3) treatment with anti-Φ serum eliminates the response of splenic T cells to PHA and Con A, but has no effect on ^{3}H-thymidine incorporation of antigen-stimulated macrophage cultures, and 4) autoradiographs clearly demonstrate a heavily labeled macrophage population. Macrophages-elicited by C. parvum, proteous peptone broth, gelatin or mineral oil failed to proliferate under identical cultural conditions, whereas DNP-BGG induced macrophages elicited by i.p. injection of agar or agarose readily incorporated ^{3}H-thymidine. Thus, it appears that the agar component of thioglycolate is responsible for the generation of a population of macrophages which do not appear to be terminally differentiated because of their vigorous proliferative response to antigen. (Operated by UCC for USDOE).

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LONG TERM HUMAN PERIPHERAL BLOOD MONOCYTE CULTURES-EXPRESSION OF HLA, Ia, AND MONOCYTE ANTIGENS. S.D. DOUGLAS[1], N.E. KAY[2], M.E. MOORE[1]*, and S. FERRONE[3]*. Children's Hospital of Philadelphia, University of Pennsylvania Medical School[1],

Phila., PA 19104, VA Medical Center[2], Minneapolis, MN 55417 and Columbia University College of Physicians and Surgeons[3], New York, NY 10032.

Human peripheral monocytes (PBM) were isolated and then cultured for periods of one month in Dulbecco's modified Eagle's medium with 10% horse-10% fetal calf serum. Reactivity with monoclonal antibodies was assessed by fluorescence microscopy using goat anti-mouse antiserum and binding to iodinated Staph. protein A. 16-36% (mean 28%) of freshly isolated PBM reacted with anti-HLA-ABC framework antibody; reactivity was similar for cells cultured for 7 days, declined after 7-21 days, and was not detectable thereafter on the monocyte-derived macrophages (MDM). 8-30% (mean 16%) of PBM reacted with anti Ia; reactivity decreased during time in culture and was not detectable after day 21. In contrast, OKM-1, a monocyte antibody, demonstrated constant binding throughout the culture period (day 1: 54-81% and day 28: 54-68% positive cells). The binding of the monocyte antibody, 63D3, to PBM and human alveolar macrophages (AM) is similar (mean fluorescence intensity values (MFI), 19 vs 16) whereas, Ia expression on AM is much greater than for PBM (MFI 9 and 262, respectively) Fed. Proc., 41:433, 1982. Thus monocyte related antigen, OKM-1, is expressed on PBM and MDM; 63D3 is expressed on PBM and AM. Ia expression is greater on AM than PBM. HLA and Ia expression decreased on MDM and are not detectable after 28 days. HLA and Ia expression on human monocyte-macrophages may be modulatd by lymphokines or other stimuli, as for murine macrophages. (Supported by the Thrasher Research Fund, and NIH-DHS Grants HL-27068 and 1P01NS-17752).

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SERUM-DEPENDENT MATURATION OF HUMAN MONOCYTES IN VITRO. R. A. MUSSON, National Jewish Hospital and Research Center/National Asthma Center, Denver, CO 80206.

Human monocytes acquire a number of macrophage functions during in vitro culture. Serum has routinely been included in these cultures. The absolute dependence of the maturation on the presence of serum was evaluated. Monocytes, were cultured in medium M-199 in the absence or presence of 0.1% to 10% autologous platelet-rich plasma derived serum. After 7 days in culture, the ability to lyse K562 cells, the intracellular level of 3 lysosomal enzymes, morphology, esterase staining (NSE), and uptake of IgG coated sheep erythrocytes (EIgG) were examined. Cytolytic activity increased 2-2.5 fold if 5% serum was included in the monocyte culture but did not increase if serum was omitted. Cytolytic activity on day 7 was dependent on the concentration of serum present during the previous 7 days (no serum, 5% killing; 0.5% serum, 21% killing; 1% serum, 26% killing; 5% serum, 30% killing; 10% serum, 31% killing). Increase in the intracellular levels of 3 lysosomal enzymes was also dependent upon the concentration of serum in the cultures (nearly maximal effect with 2% serum). NSE activity of monocytes was inhibited 90% by NaF (1.5mg/ml). This activity became fluoride-resistant during culture (100% positive cells in the presence of NaF after 3 days in 1%, 5% or 10% serum). Monocytes in 0.1% or no serum were 60% and 20% positive on day 3 in the presence of NaF. On days 1 and 2 of culture the % NSE-positive cells in the presence of NaF was dependent on the concentration of serum. Monocytes cultured in the absence of serum were predominantly a spindle shape; whereas, cells in 5% or 10% serum were predominantly a "pancake" shape. Lack of maturation, in the absence of serum, was probably not due to a general inability to function since the ability to phagocytose EIgG was not lost in serum-free cultures (71% of phagocytes active on day 0 and day 7 in the presence or absence of serum). These data demonstrate that very low levels of serum induce maturation. Identification of responsible serum components is now underway.

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HISTOCHEMICAL AND FUNCTIONAL ANALYSES OF MULTINUCLEATED GIANT CELLS DERIVED IN VITRO FROM HUMAN MONOCYTES. L. SCHLESINGER*, R. A. MUSSON and R. B. JOHNSTON JR. National Jewish Hospital and Research Center/NAC, Denver, CO 80206.

Multinucleated cells which appear at sites of chronic inflammation, are thought to arise from the fusion of mononuclear phagocytes. Little data exist regarding their function. We have compared phagocytic and metabolic activities of multinucleated and uninucleated cells derived from human monocytes after 9-14 days in culture in 10% heated (56°C) autologous serum. The percentage of cells with two or more nuclei was 32-77% ($\bar{x}$=47%, n=29 cultures from separate individuals). Reduction of nitroblue tetrazolium (NBT) dye during stimulation by phorbol myristate acetate (PMA) was equivalent in giant cells and macrophages: 75+5% of giant cells ($\bar{x}$ + SEM, n=9) and 72+5% of macrophages were clearly NBT-positive. Incubation with superoxide dismutase, reduced PMA-stimulated NBT reduction to 8-16% of cells within both populations, indicating that NBT reduction was dependent primarily on generation of superoxide anion. 14% of both cell types reduced NBT in the absence of PMA. 67+6% of giant cells ingested sheep erythrocytes (E) coated with IgG (EIgG), compared to 72+3% of macrophages (n=8). 40 to 45% (n=5) of both cell types ingested E coated with IgM and complement (EIgMC). At a ratio of 6

fungi per phagocyte, both cell types ingested an avearage of 4-6 viable Candida albicans per cell; 87.5±7.9% multinucleated cells and 89.2±5.8% macrophages ingested at least one organism (n=7). At a ratio of 2 fungi per phagocyte, the same % of ingested fungi were killed by multinucleated cells (23.5±3.9%) and macrophages (21.4±5.2%),n=7. Results to date suggest that multinucleated giant cells function like uninucleated monocyte-derived macrophages in host defense-related activities such as phagocytosis, phagocytosis-associated oxidative metabolism, and killing of candida.

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CHEMOTACTIC RESPONSES OF HUMAN MONOCYTES THAT REPOPULATE THE CIRCULATION DURING MONOCYTE DEPLETION BY LEUKOPHERESIS. ENRICA ALTERI and EDWARD J. LEONARD, Laboratory of Immunobiology, NCI, Bethesda, Maryland.

Human monocytes comprise migrating and non-migrating populations with respect to chemotaxin responsiveness. Do these differences reflect 2 cell lineages or developmental stages of one lineage? We therefore tested migratory function of young monocytes that repopulated the circulation during leukopheresis. Over a period of 2 hrs, donors were depleted of approximately 10^9 monocytes, equivalent to the total in the circulating blood; new monocytes entered the circulation, since monocyte counts were normal at the end of the procedure. Blood samples were drawn at the start and end of leukopheresis and 3 hrs later. After separation on Ficoll-Hypaque, 50,000 monocytes per well were added to multiwell chemotaxis chambers containing 4 concentrations of 3 different attractants. After 90 min at 37°C, number of migrated monocytes was measured. The number of monocytes migrating to optimal chemotaxin concentrations was decreased to about half at the end of leukopheresis, with recovery 3 hrs later. The Table shows % of pre-leukopheresis response ± SEM for 10 subjects:

	f-norleu-leu-phe	LDCF	C5a	Medium
Post-leukopheresis	52 ± 7	59 ± 5	53 ± 7	61 ± 8
3 hrs later	80 ± 13	101 ± 15	102 ± 20	96 ± 16

Since these changes were not observed in 3 sham donors who went through the same procedure without withdrawal of cells, they reflect functional characteristics of newly circulating monocytes. In 3 experiments, binding curves of fMet-Leu-[^{3}H]Phe to monocytes suggest that binding by postleukopheresis monocytes was less than that of preleukopheresis or recovery monocytes.

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IDENTIFICATION, ENRICHMENT AND PARTIAL CHARACTERIZATION OF FOLLICULAR DENDRITIC CELLS FROM MOUSE LYMPH NODES IN VITRO. C.T. Schnizlein*, K.L. Holmes*, N.L. Keesling* and J.G. Tew. Department of Microbiology and Immunology, Medical College of Virginia, Richmond, VA 23298.

Antigen-retaining Follicular Dendritic Cells (FDC) have been studied in sections of lymph nodes and spleen, but studies of these cells in culture have been limited. The purpose of this study was to isolate these fragile, highly interdigitating cells, characterize them morphologically and determine their phagocytic and adherent properties in vitro. FDC were obtained from immunized popliteal lymph nodes (PLN) of mice that had been injected in the foot pads with ^{125}I-HSA or -HRP 20 hr before sacrifice. PLN were removed 1 hr after injecting hind foot pads with collagenase. The nodes were incubated for another hr with collagenase and dispase before FDC were removed by gentle teasing. FDC were enriched up to 40-fold by density centrifugation on a BSA column where most FDC and associated radiolabel floated at densities of 1.064 g/cc or greater. Slides of the FDC-enriched fraction were prepared using a cytobucket which was less disruptive than a cytocentrifuge. Cell surface antigen was confirmed by autoradiography and also enzymatically for HRP. Wright's stained FDC had pink cytoplasm, a euchromatic nucleus with peripherally-clumped chromatin and were large (up to 60 um), irregularly-shaped cells. In contrast to resident peritoneal macrophages, FDC did not phagocytose opsonized SRBC, nor did they adhere to glass surfaces or stain with Mac-1. It appears that we have enriched for a population of non-phagocytic and non-adherent lymph node cells in vitro that fulfill the criteria of antigen-retaining FDC.

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AN INVESTIGATION OF THE "NEURAL SPECIFIC" S-100 PROTEIN AS A CYTOPLASMIC MARKER FOR DENDRITIC CELLS. G. ROWDEN, Y. YAMAMURA, T. MISRA, K. SHEIKH, E. CONNELLY, H. RIGLEY
Department of Pathology, Loyola University Medical Center, Maywood, Il 60153.

An investigation of the *in situ* localization of dendritic cells (DC) in the peripheral lymphoid organs of mice and man was carried out using PAP immunohistochemical staining of paraffin embedded tissue specimens of skin, spleen, lymph node, thymus, gut and kidney. A rabbit antiserum was employed and appropriate pre-immune and absorped sera were used to ensure specificity of staining. Besides staining glial elements in the CNS and Schwann cells in the peripheral nervous system, the S-100 protein was demonstrated in DCs in all organs examined. These were equivalent to interdigitating reticulum cells in spleens and lymph nodes, Langerhans cells in the skin, dendritic cells of the cortico-medullary junction in thymii, Peyers patches and in the interstitium of the kidney. Negative results were noted for macrophages (esterase or lysozyme positive cells) in these situations. Macrophage and DC population were isolated from mouse spleen using the density gradient and differential adherence methods of Steinman. Cytospin preparation of poly L. lysine-coated slides were stained for demonstration of S-100 antigen, lysozyme and non-specific esterase. The adherent macrophages population showed strong esterase and lysozyme activity, but little S-100 staining. The pattern was reversed for the DC population. The S-100 protein appears to be a marker for a particular subset or stage of maturation of macrophages i.e. the dendritic cell.

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MOUSE MONONUCLEAR PHAGOCYTE ANTIGENS DETECTED BY MONOCLONAL ANTIBODIES USING IMMUNOFLUORESCENCE AND COMPLEMENT DEPENDENT CYTOTOXICITY. Paul A. LeBlanc*, Shou-Mei T. Chang* and Stephen W. Russell. Department of Comparative and Experimental Pathology, University of Florida, Gainesville, FL. 32610.

We have used a panel of monoclonal antibodies to assess the heterogeneity of several commonly used mononuclear phagocyte (MP) populations. Rats were immunized with MP derived from liquid cultures of mouse (BALB/c) bone marrow. Rat spleen cells were fused to the mouse cell line Sp2/0-Ag14. Antibodies derived from the resultant clones were used for indirect immunofluorescence monitored by either conventional fluorescence microscopy or flow cytometry (FACS II) and also complement mediated cytotoxicity. Some monoclonals reacted with most MP tested, while others showed selectivity for certain populations of MP. For example, B28.1 stained most MP harvested from the peritoneal cavity 24 hr after an ip injection of LPS but only a few MP resident in the peritoneal cavity or those which were harvested 4 days after the ip injection of thioglycollate broth. All the antibodies were cytotoxic in the presence of rabbit complement. Coupling of cytotoxicity with indirect immunofluorescence allowed the investigation of population overlap. B23.1 and B25.12 stained 100 and 25% of cultured thioglycollate-elicited MP, respectively. After treatment with B25.12 and complement, which killed 25% of the cells, there was no detectable staining with B25.12 while all of the remaining cells continued to stain with B23.1. Such analysis allows the assignment of MP to subgroups on the basis of antigenic phenotype ($B23.1^+$, $B25.12^-$, vs. $B23.1^+$, $B25.12^+$). Supported in part by NIH research grants AI18133, CA23686, and CA28243.

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BONE MARROW ORIGIN OF MACROPHAGE HETEROGENEITY. I. BURSUKER*, R. GOLDMAN, Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel.

In vitro differentiation of mononuclear phagocytes from bone marrows of inflamed mice (I-BMDMP) results in mononuclear phagocyte populations which differ by various criteria from those differentiating from bone marrows of normal mice (N-BMDMP). I-BMDMP express: a) higher activity of a lysosomal enzyme marker (acid phosphatase); b) a decreased capacity to phagocytize heat killed yeast cells; c) an increased capacity to phagocytize sheep erythrocytes, opsonized with IgM and complement; d) a higher responsiveness to *in vitro* activation by macrophage activating factor (MAF) and poly-I:poly-C; e) a higher ADCC activity; f) a higher chemiluminescence activity; g) a lower sensitivity to PGE_2; h) a lower percentage of colonies expressing high 5'-nucleotidase activity. The differences between the populations are reminiscent of those observed between normal and inflammatory peritoneal exudate macrophages. Since the *in vitro* differentiation is carried out under identical conditions, the functional diversification has to originate at the level of bone marrow precursor cells. Using 5'-nucleotidase as a marker however, direct evidence

was obtained for the existence of at least two subpopulations of mononuclear phagocyte bone marrow precursor cells. Our results suggest that under normal conditions bone marrow macrophage precursor cells are inherently heterogeneous and inflammation leads to a preferential expansion of a certain subpopulation of precursor cells (i.e. those characteristic of inflammatory exudate macrophages).

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HETEROGENEITY WITHIN RAT PERITONEAL MACROPHAGE SUBPOPULATIONS. R.H.J. BEELEN*, E.C.M. HOEFSMIT*[1], W.S. WALKER. [1]Dept. Electron Microscopy, Faculty Medicine, Free University Netherlands; Div. Immunology, St. Jude Children's Research Hospital, Memphis, TN 38101

Our earlier observations on rat mononuclear phagocytes have shown that blood monocytes and (inflammatory) exudate macrophages may develop into resident macrophages via the transitional stage of exudate resident macrophages or PA-negative macrophages. However, there are considerable differences with respect to many characteristics between elicited and non-elicited peritoneal cells. We therefore would like to know whether functional heterogeneity is due to certain maturation stages or subpopulations of macrophages within the overall population. Rat peritoneal cells could be separated in this study into at least 7 distinct groups of cells (based on buoyant density) with the help of a discontinuous Percoll gradient. The macrophages (which make up ∿70% of rat peritoneal cells) were found in four discrete bands (>90% macrophages), the eosinophilic granulocytes (which make up ∿20% of rat peritoneal cells) were found mainly in one distinct buoyant density (>80% eosinophilic granulocytes) and the mast cells (which make up ∿6% of rat peritoneal cells) were found in the pellet (>80% mast cells). We observed that there were differences between the macrophage populations with respect to morphology (maturation stage), and also in their expression of Fc-receptors and Ia-antigens. Characterization of the different subpopulations with respect to other parameters (clonogenic assay, ADCC, ADP) is in progress, while the question is also in study whether these macrophage subpopulations differ only in stage of development (maturation) or stage of functional activity, or whether they may represent definite end-stages of irreversible specific differentiation.

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HETEROGENEITY AMONG PERITONEAL MACROPHAGES. E.P. MAYER AND A. GHAFFAR, Department of Microbiology and Immunology, University of South Carolina, Columbia, SC 29208.

Macrophages from the peritoneal cavity of mice were examined for heterogeneity using phagocytosis and cell size as two parameters. Cell size was measured by narrow angle light scatter on an EPICS V flow cytometer. Phagocytosis was measured by flow cytometric analysis of flourescent cells which had ingested fluorescein-labelled bacteria in vivo. The results indicated that at least two peaks of nucleated peritoneal cells could be detected by light scatter analysis. Adherence of peritoneal exudate cells before analysis removed cells mainly from the second peak, but there was also a loss of cells in the region of the trailing edge of the first peak. This observation indicates the existence of at least two subpopulations of macrophages. The phagocytic assays confirmed the existence of two macrophage subpopulations of different size. The results also suggested that the smaller macrophages may phagocytize at a lower rate than the larger macrophages. The first peak of smaller macrophages increased significantly in mice treated with Propionibacterium acnes 2 days previously but not 15 days previously. In conclusion at least two macrophage subpopulations can be detected on the basis of their size and the smaller cells may be relatively immature cells.

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DISSOCIATION OF TUMORICIDAL AND BACTERICIDAL ACTIVITIES WITHIN MOUSE PERITONEAL CELL POPULATIONS. PRISCILLA A. CAMPBELL, CHARLES J. CZUPRYNSKI AND JAMES L. COOK*. Department of Medicine National Jewish Hospital, Denver, CO 80206

Experiments were conducted to test whether populations of phagocytes which are bactericidal for E. coli and the facultative intracellular bacterium, Listeria monocytogenes

are also tumoricidal, and vice-versa. Mouse peritoneal exudate cells harvested 48 hours after injection of proteose peptone were shown to kill E. coli and Listeria in a 2 hour suspension assay for bactericidal activity. Aliquots of this same cell population could not kill TCMK tumor target cells, as measured by release of ^{3}H thymidine from the tumor cells and by visualization of tumor cell growth. Moreover, mouse peritoneal exudate cells harvested from mice injected both with 20 ug PPD 3 days earlier and with 10^{7} BCG 17 days earlier could kill TCMK tumor target cells, as described above, but could not kill E. coli or Listeria. Thus, the ability to express bactericidal and tumoricidal activity are not necessarily properties of the same population of phagocytes.

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PHYSICAL SEPARATION OF RABBIT ALVEOLAR MACROPHAGES INTO FUNCTIONAL SUBPOPULATIONS. M.A. MURPHY* and H.B. HERSCOWITZ. Department of Microbiology, Georgetown University Medical Center, Washington, D.C. 20007.

Previous work from this laboratory has demonstrated that alveolar macrophages (AM) can modulate immune responses by suppressing in vitro antibody formation and enhancing T lymphocyte-mediated functions in the form of mitogen-induced lymphoproliferation and mixed leukocyte responses. The present study was carried out to determine the role of AM in the generation of Migration Inhibitory Factor (MIF) activity. Spleen cells (SC) obtained from rabbits immunized with BCG were stimulated in vitro with PPD in the presence or absence of AM. Culture supernatants were tested for MIF activity in an agarose droplet assay using AM from nonimmune rabbits as target cells. When AM were added to unseparated SC at a ratio (1 AM:10 SC) previously shown to suppress PFC responses, the generation of MIF was generally enhanced compared to control cultures. Spleen cells depleted of macrophages by passage over nylon wool columns failed to produce MIF. Reconstitution of the depleted SC with AM restored and enhanced MIF production. To determine if the cells carrying out the diverse functions could be physically separated, the AM were centrifuged on discontinuous gradients of Percoll®. The results indicate that the less dense AM appear to function as accessory cells for the generation of MIF while those AM which suppress PFC responses appear to be located in the more dense fractions. These observations not only confirm the requirement for macrophages in the generation of MIF, but also extend previous studies which indicate that AM enhance cell-mediated responses. The results also suggest that there are functionally distinct subpopulations of AM. (Supported by NIH grant HL-25478).

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EFFECTS OF 2-DEOXY-D-GLUCOSE (2DOG) ON CHEMOTACTIC MODULATION OF NEUTROPHIL ADHESIVENESS. C.W. SMITH, J.C. HOLLERS*, D.C. ANDERSON*. Department of Anatomy, Michigan State University, E. Lansing, Michigan 48824, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030

Chemotactic factors (CF) modulate the adhesiveness of neutrophils by inducing the appearance of adhesion sites on the lamellipodia of morphologically polarized cells and by activating the transport of these sites to the cell's uropod. The effects of 2DOG on these events was investigated in isolated human neutrophils (PMN). A 20 minute preincubation of PMN in 5mM 2DOG profoundly inhibited all cellular functions normally activated by CF - chemiluminescence, increased adherence, shape change, migration and lysozyme release - as well as phagocytosis of opsonized bacteria, without influencing the specific binding of f-Met-Leu-H^{3}-Phe. The inhibitory effects were fully reversible when cells were washed and incubated 30 minutes in medium containing 5mM glucose with the following exceptions: CF failed to alter PMN adhesiveness, and migration on protein coated substrata remained profoundly depressed. Shape change, chemiluminescence, lysozyme release, phagocytosis, and bactericidal activity all returned to control levels. Albumin coated latex beads (ACLB) were used to assess the distribution and movement of adhesion sites on pretreated PMN polarized by exposure to CF. The results indicate that 2DOG causes an irreversible block in the appearance of new adhesion sites and the transport of these sites to the uropod. There appears to be a selective dissociation of these mechanisms from those involved in shape change, secretion and phagocytosis. The adhesive mechanisms appear to be important in PMN migration.

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FLOW CYTOMETRIC STUDIES OF OXIDATIVE PRODUCT FORMATION BY NEUTROPHILS: A GRADED RESPONSE TO MEMBRANE STIMULATION. D.A. Bass, J.W. Parce*, P. Szejda*, M.C. Seeds*, M. Thomas*, L.R. DeChatelet. Bowman Gray School of Medicine, Winston-Salem, NC 27103.

We have developed a quantitative assay to monitor the oxidative burst (H_2O_2 production) or polymorphonuclear leukocytes (PMNL) using a cytofluorograph, and have examined whether PMNL respond to membrane stimulation with an all-or-none oxidative burst. During incubation with normal neutrophils, dichlorofluorescin diacetate diffuses into the cells, is hydrolyzed to 2',7'-dichlorofluorescin (DCFH) and is thereby trapped within the cells. The intracellular DCFH, a non-fluorescent fluorescein analogue, is oxidized to highly fluorescent 2',7'-dichlorofluorescein (DCF) by reagent H_2O_2 or oxygen derivatives generated by glucose oxidase + glucose or xanthine oxidase + acetaldehyde; DCFH oxidation by these systems is inhibited by catalase but unchanged by superoxide dismutase. Moreover, intracellular DCFH is oxidized by PMNL stimulated by PMA. That the oxidative product is DCF, has been shown by excitation/emission spectra and by mass spectroscopy of the product from PMA-stimulated PMNL. Normal PMA-stimulated PMNL oxidize approximately 300 amoles DCF per cell. There is a close correlation between DCFH oxidation and hexose monophosphate shunt stimulation using PMNL from patients with chronic granulomatous disease or carriers of the disease. Incubation of PMNL with varying PMA concentrations caused graded responses by all PMNL present; i.e., 3ng/ml PMA caused a mean response of 60% maximal with a single population of responding PMNL (rather than 40% resting and 60% fully stimulated as predicted by the all-or-none hypothesis). In PMNL, oxidative product formation occurs as a graded response to membrane stimulation.

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RECEPTOR BLOCKADE AS A MECHANISM OF CROSS-DEACTIVATION OF HUMAN NEUTROPHILS. J. Mehta*, M. Muniain*, I. Spilberg*, L. Simchowitz*, and J. Atkinson* (INTRO by C.C. Daughaday). V.A. Medical Center and Washington University Medical School, St. Louis, MO 63106.

Deactivation and cross-deactivation represent loss of biological responsiveness of cells following brief exposure to the same or different stimulus. The present work explores the idea of receptor blockade as a mechanism of cross-deactivation by studying early molecular events of neutrophils activated by the distinct chemoattractants pepstatin and FMLP. Human neutrophils were isolated by dextran sedimentation and Ficoll-Hypaque centrifugation; chemotaxis measurements were made by the Boyden chamber technique, superoxide radical generation was measured by the cytochrome C reduction while the release of lysozyme and B-glucuronidase was used for exocytosis measurement; levels of cAMP were measured by radioimmunoassay. Pepstatin and FMLP induced superoxide radical generation, release of lysosomal enzymes, and a transient increase in intracellular levels of cAMP in a dose-dependent manner. Pepstatin competes for the ^{3}H-FMLP binding sites on human neutrophils. CBZ-PM which competitively inhibits FMLP induced neutrophil functions, inhibited superoxide generation by pepstatin but failed to inhibit C5a response. Preincubation of neutrophils either with FMLP or pepstatin inhibited, in a dose-dependent manner, the chemotactic response, superoxide radical generation, lysosomal enzyme release and change in cAMP levels when the cells were challenged with the other stimulant. Our results suggest that FMLP and pepstatin interact with the same receptor molecules to activate human neutrophil functions and that the deactivation of human neutrophils induced by these two chemoattractants is receptor mediated.

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EFFECTS OF TAXOL ON POLYMORPHONUCLEAR LEUKOCYTE FUNCTION. R.S. OSEAS* AND E. TOLOZA* (Introduced by: Michael E. Miller). Harbor-UCLA Medical Center, UCLA School of Medicine, Torrance, Ca 90509.

Taxol, a plant alkaloid derived from T. brevofolia and which promotes polymerization of the polymorphonuclear leukocyte (PMN) microtubular system, was studied for effects on the PMN responses of chemotaxis, aggregation and capping. The PMN upon stimulation by nformyl-methionyl-leucyl-phenylalanine (FMLP 2×10^{-5}M to 2×10^{-7}M) will undergo chemotaxis and aggregation. The PMN can also be shown to undergo capping using the lectin Concanavalin A and colchicine (a microtubular disrupter). All these PMN functions appear to require participation of the microtubular structure and provides the rationale for using taxol. Using a standard platelet aggregometer/re-

corder system, PMN (5 x 10^6/ml) preincubated for 30 minutes in the presence of taxol inhibited aggregation induced by FMLP at both 10^{-5}M and 10^{-7}M (FMLP 10^{-5}M 38.6 ± 3 cm^2) (FMLP + taxol 31 ± 5 cm^2). Preincubation of PMN with the microfilament inhibitor cytochalasin B (5 µg/ml) potentiated the FMLP (10^{-5}M)induced aggregation response to 54 ± 6 cm^2 , while taxol pretreatment produced inhibition to 43.0 ± 6 cm^2 of this PMN response. Using a modified Boyden chamber chemotactic assay pretreatment of PMN with taxol resulted in a 50% inhibition of chemotaxis compared to control using endotoxin activated plasma (EAP). PMN pretreated with taxol had completely normalized colchicine induced FITC-Concanavalin A capping of PMN (spontaneous capping 16%, colchicine induced 70% and taxol treated PMN 17%). Thus, taxol, a microtubular polymerizing agent in the PMN causes,inhibition of aggregation, chemotaxis and blocks colchicine induced capping and represents a new probe in the dissection of PMN structure and function.

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INHIBITION OF NEUTROPHIL PHAGOCYTOSIS BY BACTERIAL CULTURE FILTERATES. D.G. PAARLBERG*, L.A. DI PIETRO*, R.G. CRISPEN AND T.Y. SABET. College of Dentistry and Institute for Tuberculosis Research, University of Illinois, Chicago, IL 60612

Neutrophils were obtained by Ficol-Hypaque double density centrifugation of heparanized whole blood of healthy human volunteers. Recent clinical isolates; Bacteroides oralis, Fusobacterium fusoforum and two Eubacterium sp were grown anaerobically in liquid cultures. Culture media were filtered and aliquots were frozen at -70°C, and bacterial sonicates were lyophilized and just before testing were reconstituted and filtered. Phagocytosis and bactericidal capacity were assayed using immunobeads presoaked in NBT. The number of cells phagocytizing beads and those reducing the NBT were counted microscopically. When neutrophils were preincubated at 37°C for 20 minutes in presence (or absence) of bacterial preparations, washed twice then tested, none of the bacterial preparations showed inhibition of phagocytosis except a culture filterate of one of the Eubacterium sp. No effect on bactericidal capacity was observed. Various concentrations of this filterate showed a positive correlation with phagocytosis inhibition. Inhibition was reversible as recovery was completed by 25 to 30 minutes of incubation at 37°C in normal media. The inhibitor is resistant to heat (56°C for 30 minutes or boiling for 10 minutes). Alkaline treatment (pH 12 for 10 minutes) did not significantly reduce the inhibitory activity. However, trypsinization for 12 hours at 37°C eliminated the inhibitory activity. By fractionation of filterate on Sephadex G-25 the inhibitor appeared in the void volume. It is concluded the Eubacterium sp produce an inhibitor(s) that acts on neutrophils by decreasing their phagocytic activity. This inhibitory factor(s) is a high molecular weight protein that is resistant to heat and alkaline pH.

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EFFECTS OF A MODIFIED LIVE BVD VIRUS VACCINE AND ACTH ON BOVINE GRANULOCYTE FUNCTION. J.A. ROTH, M.L. KAEBERLE*. Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011.

We have previously shown that virulent bovine viral diarrhea (BVD) virus and dexamethasone are both capable of depressing granulocyte function when administered to cattle. The objective of this study was to determine if a commercially available MLV-BVD vaccine or ACTH administration caused an impairment of granulocyte function in cattle and to determine if ACTH administration potentiated the effects of the MLV-BVD vaccine. Cattle were infected with two doses of vaccine, one intramuscularly and one intranasally. Animals receiving ACTH were injected with 200 IU b.i.d. Granulocytes were separated from the peripheral blood and their function was evaluated using the following procedures: (1) Random migration under agarose, (2) Ingestion of ^{125}I-labeled S. aureus, (3) Quantitative nitroblue tetrazolium reduction, (4) Iodination, and (5) Antibody-dependent cell-mediated cytotoxicity (ADCC). The MLV-BVD virus produced a significant suppression of neutrophil iodination and ADCC. The administration of ACTH caused increased serum cortisol concentrations, an enhancement of random migration by neutrophils and a suppression of neutrophil iodination. Neutrophils from animals which received both the MLV-BVD virus and ACTH displayed enhanced random migration, enhanced S. aureus ingestion, suppressed iodination and suppressed ADCC activity. We have concluded that the modified-live BVD vaccine virus used in this study depresses granulocyte function in a manner similar to the virulent virus and that this impairment in neutrophil function may be potentiated by increased plasma cortisol concentrations.

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ASSOCIATION OF CYTOLYTIC ACTIVATION OF MURINE MACROPHAGES WITH DECREASED LEVELS OF 28S RIBOSOMAL RNA. LUIGI VARESIO, R. KOWALL. BDB, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701.

We analyzed the differences in RNA synthesis in murine macrophages that might be associated with the in vitro and in vivo activation for cytotoxicity. We observed that the activation process is accompanied by a marked decrease in 3H-uridine (^{3}H-Ur) incorporation into the RNA of macrophages. A 40-60% decrease in RNA labelling, upon pulse with ^{3}H-Ur, was observed in peritoneal macrophages (MØ) from C57BL/6 mice activated to a cytolytic stage by lymphokines (LK) in vitro or Corynebacterium parvum in vivo. To exclude the possibility that the decrease in RNA labelling was due to a dilution of the ^{3}H-Ur by an increased intracellular pool of uridinetriphosphate (UTP), we measured the specific activity of the intracellular UTP pool. The UTP pool of activated MØ had equal or higher specific activity than that of control MØ, indicating that the decrease in RNA labelling was due to reduced rate of synthesis and/or accumulation of RNA. The major change in RNA metabolism in LK-activated MØ was reduced accumulation of 32S and 28S ribosomal RNA. Little or no difference was observed in the accumulation of 18S ribosomal RNA. We concluded that the activation of macrophages for cytotoxicity is associated with alteration of RNA metabolism, and that the rate of ^{3}H-uridine incorporation into RNA may be a useful new parameter for macrophage activation.

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SPONTANEOUS CYTOTOXICITY BY HUMAN ADHERENT PERIPHERAL BLOOD MONONUCLEAR CELLS AGAINST HUMAN OR MOUSE ANCHORAGE-DEPENDENT TUMOR CELL LINES: CONTRIBUTIONS OF ADHERENT NK-LIKE CELLS. Z-L. CHANG*, T. HOFFMAN*, E. BONVINI *, H.C. STEVENSON*, and R.B. HERBERMAN. Biological Development Branch, National Cancer Institute-Frederick Cancer Research Facility, Frederick, MD 21701.

Human peripheral blood mononuclear cells (PBMNC) were found to be cytotoxic for mouse or human anchorage-dependent target cell lines in a 48-72h ^{125}Iododeoxyuridine (IUDR) release assay. Unfractionated, adherent or non-adherent cells had significant levels of cytotoxicity, as did cells fractionated according to size into "lymphocytes" or "monocytes" by elutriation. "Intermediate" size cells, not enriched for monocytes also had high levels of cytotoxicity. In all fractions tested, including adherent populations, some cells with the morphology of large granular cells were observed. Treatment of all fractions with interferon (IFLrA, purified, recombinant α-IFN) boosted cytotoxicity against four target cell lines. Treatment with lymphokines containing putative "macrophage-activating-factor" (MAF), also enhanced cytotoxicity in fractions depleted of monocytes. Culture in fetal bovine serum for six days enhanced cytotoxicity mainly in unfractionated and non-adherent PBMNC. These experiments indicate that NK-like cells may comprise appreciable contaminants in elutriator-purified monocyte-enriched or adherent cell populations and thereby contribute to observed cytotoxicity, particularly after pretreatment with IFN or other stimulatory factors.

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PHENOTYPE OF THE HAIRY CELLS OF LEUKEMIC RETICULOENDOTHELIOSIS DEFINED BY MONOCLONAL ANTIBODIES. A.J. JANCKILA,* G.T. STELZER, J.H. WALLACE, L.T. YAM,* Department of Microbiology and Immunology, University of Louisiville; The Histocompatability Laboratory, Jewish Hospital; and the Veterans Administration Medical Center, Louisville, Kentucky 40202.

Leukemic reticuloendotheliosis (LRE) is a rare form of chronic leukemia involving the proliferation of the neoplastic "hairy cells". The origin of the hairy cells (HC) is undetermined but monocyte and B-lymphocyte lineages have been proposed. In efforts to more accurately characterize HC, we examined the surface phenotype of purified HC from 4 LRE patients using a panel of monoclonal antibodies. Immunofluorescent microscopy revealed that HC from all patients displayed surface immunoglobulin (SIg) and antigens recognized by OKIa-1 and OKM-1 antibodies. HC did not express T-lymphocyte antigens. Immunocytochemistry revealed that the cells expressing these antigens were HC by virtue of their strong tartrate-resistant acid phosphatase activity. Dual-label immunofluorescence showed that most HC (75%) from all patients expressed SIg and OKM-1 simultaneously. These markers are ordinarily unique for B cells and myeloid cells respectively, and the co-ex-

pression of both HC indicates that they may be members of either cell type with defective antigen expression. Alternatively, the HC phenotype may suggest a common developmental pathway for B cells and myeloid cells of which the HC is a part.

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IMMUNOSUPPRESSIVE FACTORS IN EHRLICH ASCITES CARCINOMA FLUID INHIBIT THE MITOGENIC RESPONSE OF NORMAL LYMPHOID CELLS. J. QUDDUS AND R.E. MCCARTHY, UNIVERSITY OF NEBRASKA MEDICAL CENTER, OMAHA, NE 68105.

Cell-free Ehrlich ascites fluid (CFEAF) and factors derived from this fluid impaired the mitogenic response of normal syngeneic lymphoid cells. Fractions obtained via gel column chromatography on Sephadex-200 were highly suppressive when assayed for suppressive activity in an in vitro mitogen induced lymphocyte transformation assay using normal spleen or lymph node cells from C_3H/HeJ male mice. Fraction I, M.W. >400,000 was suppressive only for spleen cells (47% suppression), fractions II, M.W. 150,000, fraction III, M.W. 68,000 and fraction IV, M.W. <14,300 impaired the response of both the spleen and the lymph node cells by 37%-55%. Analysis of Freund's induced ascites fluid (FIAF) (used as a control) also identified 4 similar fractions with suppressive activity. Further analysis of CFEAF and FIAF by RID indicates that although CFEAF contained large amounts of IgG, no immune complexes were detectable. These results suggest that since both CFEAF and FIAF contain factors that possess the ability to inhibit T cell responses, the materials produced may be the result of an inflammatory response and not specifically induced by the tumor

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IMPAIRED CHEMOTAXIS RELATED TO ABNORMALITIES OF PMN LEUKOCYTE ADHERENCE IN PROTEIN CALORIE MALNUTRITION (PCM). D.C. ANDERSON, G.S. KRISHNA, B. HUGHES, M.L. MACE, B.L. NICHOLS, & C.W. SMITH. USDA/SEA, Children's Nutrition and Research Center, Baylor College of Medicine, Houston, TX, and Dept. of Anatomy, Michigan State Univ., Lansing, MI.

To further understand the pathogenesis of infection in PCM, serial studies of PMN motility and adherence were performed in 25 children (age 2 mos-14 yrs) with "severe" PCM ($\bar{x}$ wt/ht <3%, $\bar{x}$ pre-albumin (PA) 7.1 mg/dl). Initial assessments demonstrated diminished PMN chemotaxis in 17/20 cases; $\bar{x}$ migration scores were 64±21% to zymosan activated sera (ZAS) ($p < .001$). Chemotaxis to ZAS was further diminished when PMNs were suspended in "severe" autologous plasma (54±14% of C). In 14 nutritionally "restored" patients ($\bar{x}$ wt/ht=27th percentile and $\bar{x}$ PA=23 mg/dl), chemotactic responses were 87±14% (BCF) and 94±14% (ZAS) of C while decreased scores were constant in patients not achieving nutritional recovery. Abnormalities of PMN adherence were concurrently identified; baseline adherence values were significantly ($p < .001$) increased in "severe" PCM (49±8%) compared to C values (21±11%) or respective "nutritionally restored" values (20±12%). Also, $\bar{x}$ chemotactic factor (CF) induced adherence increments (above baseline) as seen with C PMNs (BCF, +23±14%; f-Met-Leu-Phe, +34±14%) were not observed in "severe" PCM (BCF, +0.84±14%; f-Met-Leu-Phe, -0.2±20%) ($p < .001$). In contrast, normal CF-adherence increments were observed in respective PCM patients following recovery (BCF, +25±9%; f-Met-Leu-Phe, +23±11%). Conclusion - Diminished PMN chemotaxis in PCM is causally related to intrinsic abnormalities of PMN adherence and CF mediated effects on adhesive function.

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PRESIDENTIAL AWARD OF THE RETICULOENDOTHELIAL SOCIETY

President John Spitznagel is pleased to announce cash awards and plaques for the best student papers presented at the Nineteenth National Meeting of the Reticuloendothelial Society. There will be one competition which will include all "candiates in training" (predoctoral and postdoctoral) with a maximum of two years of postdoctoral work. These awards are supported, in part, by funds provided by the Annie R. Beasley Memorial Fund. Winners will be announced at the annual banquet, Tuesday evening, October 19, 1982.

The co-winners of the 1981 awards were:

Mr. Steve J. Noga	Ms. Elizabeth A. Weaver
Dept. of Pathology	Dept. of Microbiology
University of Florida	University of Texas
Gainesville, FL	Galveston, TX

THE MARIE T. BONAZINGA AWARD

The Reticuloendothelial Society will present the Marie T. Bonazinga Award at the 19th Annual National Meeting. This award is sponsored by the Accurate Chemical and Scientific Corporation and is to be presented to a member of the Reticuloendothelial Society who has demonstrated excellence in Research. Presentation of the $500 award will be at the banquet on Tuesday, October 19, 1982.

IN MEMORIAM

Dr. Gustavo Cudkowicz died in May 1982. He was an active member of the Reticuloendothelial Society and played an important role in its growth. He chaired the Publications Committee and was honored by the Society in 1980 with the first Marie T. Bonazinga Award for his work on the role of Natural Killer cells in foreign hemopoietic and leukemia grafts. We will miss him and remember him as a dedicated scientist and close friend.

Dr. Lawrence R. DeChatelet died in June 1982. He was a highly productive contributor to knowledge covering oxidative metabolism of human neutrophils and an enthusiastic supporter of the Reticuloendothelial Society. He served on several committees, the editorial board of the Journal and was a member of the Council. We all join in mourning for this close friend and splendid young scientist.