AD-A184 993 STUDIES OF ALTERED RESPONSES TO INFECTION INDUCED BY THERMAL INJURY(U) CALIFORNIA UNIV SAN FRANCISCO C L MILLER 01 MAY 85 DAMD17-82-C-2001 1/1

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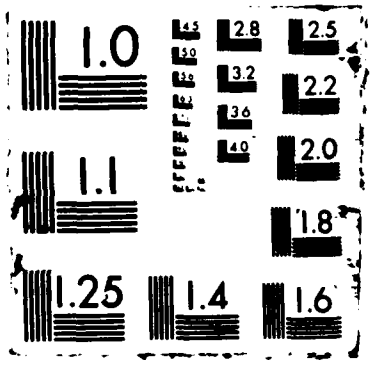
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STUDIES OF ALTERED RESPONSES TO INFECTION INDUCED BY THERMAL INJURY

ANNUAL PROGRESS REPORT

Carol L. Miller, Ph.D.

April 30, 1984 - May 1, 1985

Supported by

U.S. Army Medical Research and Development Command

Fort Detrick, Frederick, Maryland 21701-5021

Contract No. DAMD 17-82-C-2081

University of California
San Francisco, California 94143

SECURITY CLASSIFICATION OF THIS PAGE

AD-A184 993

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; Distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Univ. of California 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) San Francisco, California 94143	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD 17-82-C-2081
8c. ADDRESS (City, State, and ZIP Code) Ft Detrick Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62772A PROJECT NO. 3S1-62772A874 TASK NO. AD WORK UNIT ACCESSION NO. 127

11. TITLE (Include Security Classification)

Studies of Altered Response to Infection by Thermal Injury

12. PERSONAL AUTHOR(S)
Dr. Carol L. Miller

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 4/30/84 TO 5/1/85	1985 May 1	24

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	13		thermal injury, monocyte function, monocyte T-Cell interaction
06	05		

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

The data which we have accumulated in this contract period have provided information not only on patient MØ defects but also on human MØ-T Cell interaction pathways in general. We have demonstrated that MØ PA synthesis is a property of a MØ subset separate from the inh MØ and distinct from at least one LP producing MØ subset. We have shown that T_s through their action on monokine production could affect many host defense systems and we have indicated that a MØ defect may be crucial in trauma-induced immunoincompetence.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia M. Miller	22b. TELEPHONE (Include Area Code) 301-663-7325	22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

FORWARD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

For the protection of human subjects, the investigator has adhered to policies of applicable Federal Law 45CFR46.

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Introduction

Sepsis is the major cause of mortality following severe injury. Immunoincompetence, involving both augmented T_s activity and increased inh MØ function, plays an important role in the increased risk of septic complications of post-trauma patients.

Both the constituents of the non-specific inflammatory system and the components of the specific immune system are regulated by MØ and their products (monokines). Consequently, aberrations in MØ function could account for many of the defects in host defense seen in trauma patients. Additionally, MØ are pivotal in the balance between generations of regulatory lymphocytes and the induction of classical immune lymphocytes. Trauma appears to mediate the unbalancing of the immune response toward excessive regulatory cells. This trauma mediated derangement of MØ function could cause disruption of metabolic control, hypercoagulability, loss of PMN activity, and altered activation of the plasma protein-effector system.

The objective of our studies is to define those mechanisms by which trauma could induce excessive regulatory cells and to develop prophylactic treatment which may mitigate adverse trauma-induced MØ-T_s dysfunctions. We have, therefore, concentrated our current investigations into characterizations of the types and nature of MØ aberrations which develop after trauma. In particular, we are focusing on those MØ dysfunctions which could contribute to the development of excessive regulatory activity.

There is a variety of different MØ dysfunctions that could develop after trauma results. The heterogenous MØ population contains subpopulations whose products include plasminogen activator (PA), complement components (C), tissue procoagulant factors (TF or PCA), and varieties of monokines such as leukocyte

pyrogen (LP, . .cerleukin 1 (IL-1), neotrophil activating factor (NAF), and prostaglandin E_2 (PGE_2). These monokines act by either stimulating or inhibiting protein synthetic pathways of other leukocytes and are probably products of different MØ subsets.

The various MØ products mentioned above have all been shown to be affected by trauma result. Consequently, we focused on altered fac MØ function, as measured by changes in monokine production, as a likely initiator of altered MØ-T cell interaction post-trauma. We chose plasminogen activator (PA) production as our measure of fac MØ function. PA could be measured in a T-cell free assay system. T-cells in the system confuse MØ mediated defects with T-cell mediated defects which are also reflected in MØ. PA was also known to act as an immune mediator with mitogenic activity for T_h (1-4). We demonstrated that depressed MØ PA function appeared several post-trauma days before detectable T_s and that PA depression correlates both to mitogen hyporesponsiveness and increased septic complications (5-7). We interpreted these data as indicating that a crucial MØ dysfunction initiated immunoincompetence.

Other investigators, finding no defect in post-trauma MØ IL-1 production, concluded that a MØ defect played no role in initiating post-trauma immunoincompetence (8, 9). Unfortunately, the IL-1 assay gives no information on MØ Ag presenting capacity. In addition, the technique used to test IL-1 (the LAF assay) measures monokine activation of all T-cell subsets since it assesses thymocyte proliferation. Therefore, the LAF assay would fail to detect a shift in monokine type from T_h activating (IL-1) to T_s activating. Both PGE_2 and other inhibitory monokines are known to preferentially increase T_s proliferation (10, 12-16). Several laboratories including our own, have shown an increased PGE_2 production occurring almost immediately post-trauma (7, 17-19). Therefore,

the detection of unaltered LAF activity post-trauma, rather than indicating normal fac MØ function, may be indicating aberrant MØ activation of T_s. Furthermore, MØ generation of LP activity generally parallels production of IL-1 activity, but some MØ supernates with high LP activity are involved in T_s generation (11, 20-22). Our data indicate increased MØ LP activity in trauma patients who develop septic complications.

Other MØ secretory products like complement components and PCA also have potent effects on the immune system (23-33). Synthesis of these MØ products is also altered post-injury (34-36). C synthesis decreases while PCA generation increases (34-36). All these monokine changes may reflect alterations in MØ subset ratios and consequent changes in MØ-T cell interactions. However, since our isolated MØ populations contain both inh and fac MØ, these data could result from increased patient PGE_2 activity rather than from a fac MØ defect. In order to actually pinpoint the early events which unbalance the immune network, it is necessary to examine separated patient MØ and T lymphocyte subsets. Although recent development of monoclonal antibodies and the availability of sorting techniques have made such isolation feasible, there are still some problems in producing an absolutely purified T-cell or MØ subset. Preparation and functional analysis of purified MØ and T-cells is impractical as a daily monitoring system for trauma patients.

Post-trauma depression of monokine activity could reflect a change in the MØ antigen presenting cell (APC) subset rather than increased inh MØ in the test population. A T_s subset which suppresses MØ Ia expression has been detected in murine and human systems (37, 38). The appearance of such a T_s subset after trauma would severely compromise facilitory MØ function and eventually generate more T_s (perhaps another subset). A T_s with a MØ target has not as yet been detected post-injury. Such a T_s subset could have gone undetected because it

was genetically restricted, it had only a MØ APC target, and/or its effect was solely detectable on target cells with increased susceptibility to suppression (patient's own cells) (39-42). Current assays for trauma patients' T_s assess suppression of third party allogeneic MLR or mitogen responses (43-46). Neither of these systems detect genetically restricted T_s. The mitogen assay would fail to detect a T_s with an APC MØ for a target. Based on recent murine data characterizing MØ-T_s interactions, we conclude that the appearance of a genetically restricted T_s immediately post-trauma is probably not the initial trigger of immune unbalancing. (Genetically restricted T_s subsets require MØ Ag presentation in the context of an I-J gene product [10, 47-49]). Removal of I-A positive murine MØ increases both the proportion of I-J bearing MØ and the generation of non-genetically restricted T_s which can be induced directly by Ag. Consequently, the reduction of facilitory MØ is the primary cause of increased T_s generation, whether acting thru direct T_s interaction with Ag, or thru increase of I-J bearing MØ T_s and inh MØ are interdependent as are T_h and facilitory MØ. The genotype of the Ag presenting MØ also determines the genetic restriction for later T-cell interactions with other T or B cells (47, 50-52). PGE_2, the product of inh MØ, expands T_s generation in much the same manner as IL-1 expands T_h generation (14-16). The induction of many T_s subsets in disease pathology occurs secondary to MØ aberrations (47, 53-57). Although initial appearance of T_s is an unlikely trigger of post-trauma immunoincompetence, T_s which suppresses APC may play an important role in maintaining fac MØ depression. We therefore need to evaluate increased sensitivity of MØ to T_s as well as to examine MØ Ag presenting capacity and MØ induction of T_s when defining post-trauma alterations in MØ-T cell interactions.

There is another possible complication in evaluating post-trauma alterations in patients' MØ-T cell interactions. Many MØ adjuvants such as MDP, LPS, and

peptidoglycan are bacterial products which would be present in the local wound environment after severe injury. Such MØ stimulators might change the state of MØ activation and thereby alter the MØ-T cell interactions at the local site. Peripheral blood MØ-T cell interactions may not reflect MØ activity at local sites. Therefore, it's important to assess, post-trauma, not only monokine production and MØ-T cell interaction, but also MØ responses after adjuvant stimulation. It is necessary to demonstrate that the detected post-trauma alterations in monokine production and/or MØ-T cell interactions are stable physiological changes irreversible by adjuvants. This does not mean that giving adjuvants immediately post-injury could not prevent a MØ defect but only that a trauma-generated MØ defect is stable.

METHODS:

Normal volunteers (medical staff) are utilized as donors of control human leukocytes. Consenting asplenic trauma patients are assigned an ISS score. Data from these patients' assays are assessed by comparing ISS scores of 9-25, 25-35 and scores >35. In this manner, similar degrees of trauma can be assured when patient responses are compared.

All patients assayed are first drawn on admission and then drawn again every 3 days until release or demise. Approximately 20 ml of blood is collected on each assay day. MØ are isolated from the peripheral blood (PBL) by Ficoll-Hypaque gradient centrifugation. The isolated cells are simultaneously tested in the PHA, PA, TF, mitogen, PGE_2, C, LP and lysozyme assays. Clinical status of the patients is evaluated by co-investigator every three days and the information incorporated into the patients' data summary.

We monitor the ability of patient and normal MØ to respond to phytohemagglutinin (PHA). This non-specific mitogen response requires the cooperative interaction of MØ and T cells. The isolated MØ are routinely

examined for the production of PA, TF, PGE_2 and their synthesis of lysozyme. In the PA assay, patients' and normal controls isolated MØ are placed into ^{125}I-fibrin plates and cultured 18 hours either in the presence of acid treated fetal bovine sera (AT-FBS) or soybean trypsin inhibitor (SBTI), an inhibitor of plasmin. After all the PA is released in cultures, the cells are washed and fresh AT-FBS or SBTI media are added for another 24 hr. incubation period. The amount of fibrinolysis is adjusted to produce approximately 25-35 fibrinolytic units for normal individuals (4×10^5 isolated MØ). TF production is measured using the Rickle's assay and lysozyme production is measured by the Schill and Schumacher lysozyme plate test.

Samples used in the PGE_2 and LP assays are obtained from the MØ supernates of trauma patients and normal control. We are utilizing a modification of the classic radioimmunoassay for PGE_2 as described by Wahl (58). LP assay was assessed by using a minor modification of the method previously described by Bodel and Miller (59).

Only selected patients (those judged on clinical evidence to be at high risk) are assessed for T_s activity. T cells were isolated and/or depleted from PBL by rossetting with neuraminidase-treated sheep erythrocytes (60, 61). Enrichment or depletion of T cell subsets was done by treatment with commercially available monoclonal antisera (Ortho Pharmacentical) and the Fluorescence Activated Cell Sorter (FACS). FACS sorting involves a positive selection of suppressor cell subpopulations by means of FACS and fluorescinated specific anti-T cell monoclonal antibodies such as OKT8 and OKT4. We are also further segregating the $OKT4^+$ suppressor inducer utilizing Ortho monoclonal antibody OKT17. Both patient cells and ConA generated cells are sorted and assayed for their effect on MØ production of PA or T cell activity.

MØ complement production is measured by using a modification of the classic hemolytic plaque assay (62). Adherent purified MØ monolayers are prepared by layering cell suspensions onto glass coverslips contained in a petri dish. The coverslips are placed on a thin layer of solidified agarose in a small petri dish. The indicator cells EAC_{14} (for C_2 PFC) are added to an agarose solution and poured over the coverslips in the petri dish. The dishes are then incubated and the C_2 PFC are revealed by adding the EDTA-treated rat complement.

RESULTS AND DISCUSSION:

Monitoring of patient PA, PCA, and PHA responses

During this contract period, we studied 40 patients (12 burn patients and 28 trauma patients) whose medium age was 26. Two burn patients and one trauma patient succumbed to sepsis. None of the trauma patients with ISS score of 10-22 developed any immune dysfunction. Eighteen trauma patients, splenectomized (spx) because of their injuries, were studied in a protocol to assess dextran as a modulator of immune functions. Of these spx trauma patients, 10 had ISS scores of <25. All 8 of the spx trauma patients with ISS scores >25 developed immune dysfunction, 6 of these patients also experienced infectious complications. Table 1 illustrates the altered MØ and/or T-cell functions detected in the 12 burn patients. Table 2 represents the data from the dextran study of trauma patients. As we have previously published, depression of MØ PA production and mitogen hyporesponsiveness were positively correlated in these studies. The data also illustrate that trauma selectively affects MØ functions. While MØ PA production is decreased, MØ PCA generation is augmented. MØ lysozyme production is either unaltered or slightly increased (data not shown). MØ PA depression appears 2-4 days earlier post-trauma than does mitogen hyporesponsiveness and persists even when the mitogen response has returned to normal levels. These data are consistant with our hypothesis that

TABLE 1

BURN PATIENT IMMUNE PROFILE

GROUP III

Patient	Age	Max % PA sup	Max % PHA	% Burn	Outcome
Ge	81	67	-61	25	Fatal pseudomonas pneumonia day 12 post burn
Cr	86	55	-74	15	Recurrent strep infectious discharged day 126
Ma	47	58	-53	35	recurrent 3 taph infectious discharged day 58
Ba	76	62	-94	17	Fatal E. Coli sepsis day 25 post burn
Be	58	64	-78	25	Strep pneumonia sepsis discharged day 91
		GROUP II			
Gr	21	49	+280	40	Strep infect recovered
Ta	28	40	+128	39	pseudomonis infect
Nu	46	40	167	35	Staph infection recovered
		GROUP I			
We	22	10	7	34	No complications discharged day 20
Ou	23	20	12	20	No complications discharged day ---
Ba	64	10	4	12	No complications discharged day 42
Mu	39	30	17	60	No complications discharged day 39

TABLE 2

Altered MØ and Mitogen Responses After Trauma

Asplenic Trauma Pt

ISS	Δ PA	Δ PHA	Δ MAX PCA
29	26.7 → 8.4	- 55	+ 50
34	32.0 → 11.7	- 80	+ 30
34	22.0 → 4.3	- 75	+ 49
34	22.7 → 7.4	- 68	+ 57
34	23.7 → 9.4	- 65	+ 35
41	21.7 → 12.7	- 58	+ 30
43	21.6 → 8.8	- 78	+ 45
57	27.0 → 1.0	- 80	+ 37

Splenic Complete Trauma Pt

ISS	PA	PHA	MAX PCA
25	24.6 → 12.2	- 42	+ 26
26	17.8 → 3.2	- 84	+ 40
26	37.3 → 3.4	- 89	+ 32
29	22.4 → 13.1	- 31	+ 33
29	30.9 → 4.2	- 85	+ 50

Splenic Complete Trauma Pt

ISS	Δ PA	Δ PHA	Δ MAX PCA
29	25.6 → 8.2	- 52	+ 64
29	23.1 → 10.2	- 32	+ 38
29	23.3 → 7.8	- 52	+ 36
29	26.6 → 9.7	- 90	+ 30
30	21.0 → 8.0	- 70	+ 25
30	31.6 → 8.5	- 82	+ 36
34	28.9 → 11.8	- 80	+100
34	28.6 → 11.7	- 41	+ 42
41	26.6 → 3.8	- 78	+ 40
41	17.8 → 6.8	- 71	+ 37
43	27.2 → 7.8	- 72	+ 60
43	27.0 → 7.8	- 68	+ 34
50	36.7 → 10.1	- 57	+ 48
50	35.6 → 12.3	- 46	+ 32
50	20.2 → 6.0	- 64	+ 42

severe injury alters crucial MØ functions and that these MØ alterations trigger further aberrant MØ-T cell interaction. If trauma-mediated changes in crucial MØ functions are extensive enough, not only immune function but also other host defense systems such as neutrophile chemotaxis and phagocytosis could be critically depressed.

Evaluation of MØ LP activity

While our own data seemed to suggest that trauma mediates a facilitory MØ defect, other investigators' results detected no decrease in MØ production of IL-1 as measured in the LAF assay (8, 9). Leukocyte pyrogen and IL-1 appear to be two different activities of a related biological moiety (20-22). However, it also appears that there may be more than one molecular compound that has both LP and T-cell mitogenic capacity (i.e., IL-1 activity). One of these LP/IL-1 moieties may be inducing T_s expansion and proliferation rather than T_h proliferation (11). We initiated a modification of the Bodel leukocyte pyrogen assay. This assay uses mice as the test animal and reveals all pyrogenic activity of MØ supernates. We have examined the MØ supernates of PA hyporesponsive patients for their production of LP.

Contrary to our expectations, we found that PA depressed patients who later experienced septic complications actually had increased LP (Table 3). The increased LP activity was maximal 5-8 days post-injury. This is a period when increased T_s are also detectable.

Measurement of patient MØ PGE_2 synthesis

PGE_2 is another monokine which is known to expand T_s generation (14-16). Excessive PGE_2 levels also can directly suppress MØ function, lymphocyte function, and PMN maturation. Consequently, an increase in PGE_2 levels could be a primary trigger of many of the alterations seen post-trauma. If elevated PGE_2 levels are a major contributor to post-trauma immunodepression, then specific

TABLE 3

Elevation of MØ LP Production Concominant to Depressed MØ Immune Function and Unaltered MØ PCA Activity

	4 - 6 Days Post-Injury		
	LP Levels	PA Production	PCA Activity
Septic Trauma Patients	+.86 ± .17	9.6 ± 2.6	17.4 ± 6.7
Trauma Patients	+.33 ± .12	34.5 ± 5.0	2.9 ± 7.7
Controls	+.27 ± .12	31.4 ± 5.8	5.6 ± 4.2

treatments (such as indomethacin which is antagonistic to PGE2 synthesis) might reverse some or all of the trauma-induced immunodepression. We have encountered some problems with the commercial PGE_2 RIA kit we initially used to measure PGE_2 levels in patient MØ supernates. This kit required an extensive extraction procedure followed by conversion of PGE_2 to PGB. Both procedures have low efficiency and a highly variable product recovery. As a consequence of these technique problems, our accuracy in quantifying PGE_2 amounts was poor. When we ran different known quantities of PGE_2 through our assay system, we found that we could not detect amounts less than 15,000 pg and that we could not discriminate 50,000 pg from 100,000 pg. This means that when we detected 40,000 pg in patient samples using the commercial H-PGE_2 kit, the actual PGE_2 levels were much higher. In addition, patients' MØ supernates which were assessed as having PGE_2 levels of less than 200 pg probably had at least 10,000 pg. This insensitivity is probably the reason why patient PGE_2 production seemed to appear and disappear rather than to progressively increase and decrease. We are now employing a more specific anti-PGE_2 antibody and a purified ^3H-PGE_2. We have developed our own assay using these specific reagents with a modification of Wahl's assay (58). We now have this improved assay functioning routinely. The difficulties encountered performing the RIA assay resulted in only the PGE_2 levels of burn patients' MØ supernates being evaluated during this contract period. These data presented in Table 4 show that only patients who later developed severe septic complications showed elevated PGE_2 levels at 1-4 days post-injury (Group III patients). Interestingly, Group II patients showed elevated PGE_2 levels late in their clinical course after their infectious episode. This late rise in PGE_2 may reflect a natural mechanism to shut down the hyper-immune response (elevated PHA) that is characteristic of Group II patients. After the infectious challenge has been handled, the normal

TABLE 4

MØ PGE_2 Production and PA Activity of Burn Patients at 1-4 Days Post Injury

Pt	Group	ΔPA	$\Delta PGE_2 \times 10^{-3}$ pg	Outcome
Cr	Group III	31.8-14.7	2.6-11.1	Recurrent staph sepsis
Be	Group III	41.6-15.0	5.3-12.5	Strep sepsis
Nu	Group II	25.0-18.9	6.7- 9.9	Staph infection, no complications
Gr	Group I	44.2-39.9	5.8- 5.0	One wound infection, no complications

regulatory mechanisms may decrease the responses to original levels in these patients. We expect to expand these experiments to assess trauma patients' MØ PGE_2 synthesis within the next several months. Experiments examining MØ supernates from one severely injured individual (ISS 43) have shown massively increased PGE_2 levels at 3 days post-injury. This individual went on to develop pneumonia. Besides characterizing the MØ PGE_2 levels in the trauma patients, we will enrich for DS negative cells and evaluate whether increased PGE_2 is due to augmented numbers of PGE_2 producing MØ or increased PGE_2 synthesis per inh MØ. If we determine that augmented MØ PGE_2 production is a major factor in post-trauma immunoincompetence, then we could evaluate indomethacin treatment in one of our animal models. In addition, we would evaluate the ability of the PGE_2-containing patient MØ supernates to augment the generation of T_s.

Examination of T_s-MØ interactions

Once T_s have been generated post-trauma, their main inimical effect may be to further depress crucial MØ functions. In this manner, a trauma generated T_s would maintain the immune imbalance in favor of regulatory cells and also alter other non-immune host defense systems. For example, if T_s can suppress MØ C synthesis, they could ultimately affect neutrophile function. We have already demonstrated that T_s can suppress MØ PA synthesis. In our analysis of MØ-T_s interactions, we have been able to demonstrate that both the $OKT8^+$ T^s and the $OKT4^+$ $OKT17^+$ T_s can suppress MØ function (Table 5). Our normal MØ to Con A induced cell ratio is 2:1. However, in some experiments, the Con A induced cells were depleted of $OKT8^+$ cells by antibody plus complement or enriched for $OKT8^+$ cells by FACS sorting. In these experiments we altered the ratio to 10:1 because we recovered a much smaller Con A induced population after depletion or enrichment. As can be seen in Table 5, at this greatly reduced concentration, the total Con A induced population was only marginally suppressive. However,

TABLE 5

Suppression of MØ PA Production by Con A Activated Cells PA as % Fibrinolysis

Exp#	Control	Control MØ + Total Con A Induced Cells (% sup)[1]	Control MØ + OKT8$^-$ Depleted (% sup)[2]	Control MØ +OKT8$^+$ Enriched (% sup)[3]
321	46.5	25.2 (46%)	35.1 (25%)	N.D.
387	79.5	46.1 (42%)	60.1 (25%)	N.D.
399	37.8	17.5 (54%)	23.7 (27%)	N.D.
400	37.8	20.5 (46%)	32.9 (13%)	N.D.
418	49.8	30.2 (39%)	43.5 (13%)	N.D.
422	49.7	37.9 (24%)[4]	34.6 (30%)	37.9 (24%)
430	26.3	13.3 (48%)	20.2 (23%)	21.7 (17%)
432	62.3	52.3 (17%)[4]	49.6 (20%)	42.4 (32%)

[1] % suppression mediated by a ratio of 2 MØ/1 Con A induced cell

[2] Con A induced cells treated with OKT8 + C (OKT8$^+$ cells depleted)

[3] Con A induced cells FACS sorted for OKT8$^+$ cells (OKT8$^+$ enriched)

[4] Suppression ratio changed 10 MØ : 1 Con A induced cells

the T_s enriched populations were quite suppressive. It is in these experiments that we discovered that the $OKT4^+$ population was often as suppressive as the $OKT8^+$ population. In experiments currently in progress, we are verifying that the suppressive activity in the $OKT4^+$ T-cell population are due to the $OKT17^+$ suppressor inducer. We have also shown that T_s which suppress MØ PA generation do not depress MØ PCA or lysozyme activity. We have also been able to show that MØ whose PA response is significantly suppressed by T_s action show greatly elevated LP production (Table 6). These results are important because they not only show that MØ activity is selectively affected by T_s (i.e., PA down, LP up), but also they imply that T_s can induce or increase MØ IL-1/LP activity just as T_h do. The important question to be resolved is whether the LP/IL-1 activity induced by these T_s can mediate proliferation by the T_h clone (D10.GA.1). If T_s can induce an IL-1 that will activate T_h, it implies that the critical human MØ-T interaction which determines if T_s or T_h are activated is at the level of Ag presentation just as it is on the murine system. We interpret our data as indicating that Ts selectively inhibit MØ function. In addition, we suggest that these data support our premise that MØ PA production is an activity that also characterizes the APC MØ subset. It has been demonstrated that T_s can suppress the fac MØ subset without affecting inh MØ subsets (38, 63-66).

Experiments examining MØ stimulators

After severe injury, the microenvironment is likely to contain a number of bacterial products and bacterial cell wall fragments. Many of these materials can have very potent effects on MØ function. Many such bacterial adjuvants are able to non-specifically increase MØ activation. Consequently, even though we detect a dysfunction in peripheral blood MØ function, this dysfunction may be modulated by adjuvants at local injury sites. Alternatively, if a MØ's response to adjuvants depends on its differentiation state, then trauma patients' MØ may

TABLE 6

Suppression of MØ PA Concominant to Enhanced LP Production

		% Sup	LP
$\bar{x}$ Norm	MØ alone	0	.2
Exp.1	MØ + T_s	63	1.1
Exp.2	MØ + T_s	43	.7

respond differently to adjuvant stimulation than normals since their differentiation state may be altered. In preliminary experiments, we have characterized the effect of purified peptidoglycan (isolated and kindly supplied by Dr. R. Mishell's laboratory) on normal human MØ responses. As can be seen in Table 7, peptidoglycan (PEP) both soluble and insoluble PEP was inhibitory to MØ PA production. The insoluble PEP produced much more striking inhibition, however. We also compared purified soluble peptidoglycan to less pure insoluble peptidoglycan. Our results were that both types of PEP depressed PA production, enhanced LP production and insignificantly affect PCA and lysozyme activity. Selected samples were assessed in the lymphocyte activating assay (LAF). The LAF titre of these samples were increased 3-6 fold. Antigen presenting capacity (APC) of these MØ was decreased. These data are particularly interesting because they mirror our trauma patient results. That is, PA synthesis was decreased concomitant to increased LP activity. We suggest that the LP activity in these peptidoglycan-treated MØ supernates may be reflecting either a lymphocyte activating factor (LAF) activity for T_s or that IL-1 production is unrelated to MØ APC defects. In addition, the ability of peptidoglycan to selectively depress one MØ function (PA) while augmenting others (LP/LAF) indicates that at least two stable MØ subsets are concomitantly present in normal MØ populations. We are repeating these experiments using a DQ negative MØ population. If we can absolutely correlate MØ PA production with an APC MØ subset, it will make monitoring of patient immune function immensely easier.

TABLE 7

Differential Peptidoglycan Effect on MØ Function

Insoluble PEP						Soluble PEP					
PA % fibrinolysis		LP temp		PCA		PA % fibrinolysis		LP temp		PCA	
Norm	+PEP	Norm	+PEP	Norm	+PEP	Norm	+PEP	Norm	+PEP	Norm	+PEP
39.9	11.3	.5	.7	3.0	4.8	33.0	20.	.38	.6	6.7	13.4
31.1	17.7	.3	1.1	1.8	1.0	39.9	25.4	.5	.6	3.9	5.6
33.2	12.7	.1	.75	3.2	4.3	33.2	23.7	.2	.5	3.2	3.1
16.9	2.7	.1	1.8	9.3	12.3	16.9	9.6	.1	.5	9.3	11.2
14.5	4.0	-	-	3.9	2.8	32.3	17.7	.1	.4	3.9	3.1
43.1	7.2	.1	.6	14.0	16.5						
28.7	7.9	.2	.87	14.2	7.6						

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