AD_____

STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY SEVERE INJURY

ANNUAL PROGRESS REPORT

CAROL L. MILLER, PH.D.

MARCH 1, 1988

JANUARY 24, 1987 - JANUARY 23, 1988

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-86-C-6097

University of Massachusetts

Worcester, Massachusetts 01655

SECURITY CLASSIFICATION OF THIS PAGE

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: University of Massachusetts; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Worcester, MA 01605	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: Contract No. DAMD17-86-C-6097
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62772A	3S16 2772A874	AD	127

11. TITLE (Include Security Classification): Studies of Altered Response to Infection Induced by Severe Injury

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

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual Report	FROM 1/24/87 TO 1/23/88	1988 March 1	17

16. SUPPLEMENTARY NOTATION

Keywords: Monoclonal antibodies

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	13	
06	05	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): Monoclonals, Recombinant DNA, Thermal Injury, Infection, Antibody, Leukocytes, Guinea Pigs, (K⁻).

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

Twenty-six patients have been studied during this contract year, including 14 trauma and 12 burn patients. Of these patients, 2 burn and 4 trauma patients have succumed to fatal sepsis. Patients showed depressed moncyte (MØ) plasminogen activator (PA) activity and depressed PHA induced T cell proliferation concomitant with elevated MØ prostaglandin E_2 (PGE_2) production, as well as increased tumor necrosis factor (TNF) activity and poor prognosis. A post-trauma shift in the ratio of MØ subsets towards inhibitory MØ appears to be causal in development of these aberrant MØ functions. A new bioassay for determination of TNF has been developed this year, and a ELISA technique has been established to detect the MØ PGE_2 production. The thermally injured guinea pig model has been applied to pursuing the new prophlactic therapies. In particular we are examining cyclo-oxygenase inhibitor effects on TNF in this model. Overall, this has been a highly productive year. Several other new methods to monitor immunosuppression developed in severely injured patients are being established including an assay for Interleukin-6 and a bioassay for Transforming Growth Factor β. Several prophlactic drug trials are in progress and we are examining the monocytes from trauma patients receiving ibuprofen and the v-5 antibody against endotoxin.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mrs. Virginia Miller	301/663-7325	SGRD-RMI-S

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

FORWARD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

TABLE OF CONTENTS

Introduction

The purpose of this research is: (1) to define the role of monocyte and T cells and their products - different cytokines in mediating immunosuppression in post trauma patients; (2) to develop and improve assays for assessment of MØ functions and to detect different monokine productions; (3) to study the relationship between immunosuppression and post trauma alterations in MØ subsets, and (4), to investigate prophylactic modalities for reversing immunosuppression.

During this contract year, we have made significant advances in pursuing these goals. First, we have demonstrated that trauma induced suppressor T cells (T_s) can depress MØ functions, and this T_s mediates immunosuppression by inducing a shift in the MØ subsets toward a subset with augmented prostaglandin E_2 (PGE_2) producing capacity, as well as an increased tumor necrosis factor (TNF) activity. Second, we made tremendous progress in goal two; we have initiated the bioassay for measurement of MØ TNF production. TNF is known to be involved in cachexia and its elevation is associated with endotoxin shock. The new established method allows us to detect an increased TNF level in immunosuppressed patients. We have also successfully switched our PGE_2 assay from an RIA to a more sensitive ELISA. This ELISA is more reproduceable from assay to assay. We are using a densitometer which reads the ELISA plates and transfers the data directly into our Compaq computer. As mentioned above, we have shown that elevation of MØ PGE_2 and TNF production appears to correlate with a shift towards a MØ subset that can be identified by its expression of the high affinity receptor for human IgG1, human IgG3, and mouse IgG2a. This shift in MØ subpopulations corresponds to an increase in these patients' MØ production of PGE_2 and TNF consistent to a depression of their antigen presenting function and plasminogen activator activity. This is the reason that we have pursued goal three. If we can associate a change in MØ function with a change in MØ phenotype, the increased appearance of this particular subset could then quickly detect the onset of immunoincompetence in trauma patients. Such a quick and easy detection of immunoincompetence would have application to identification and segregation of combat victims requiring more intensive care. Our data have demonstrated that there is an increase in the number of $FcRI^+$ MØ subset and that this increase correlates both to an elevation of PGE_2 and TNF production and poor clinical outcome. Finally, in pursuing the goal of testing new prophlactic therapies, we have received animal experimentation approval for the thermally injured guinea pig model and have performed several experiments using this model. We intend to examine the effect of ibuprofen on the development of increased MØ TNF, PGE_2 production and decreased MØ PA activity. We have examined the effect of the MØ stimulator, muramyl dipeptide (MDP), on patient's MØ function *in vitro*. The MDP stimulated MØ still exhibited depressed PA activity and elevated PGE_2 and TNF production. This MDP mediated

result is similar to the suppressive effect we see mediated by early patient Ts. The MDP model may be relevant to bacterial product mediated suppression and Ts mediated suppression in these patients. When indomethacin was added to the MØ cultures simultaneously with MDP, the augmented PGE_2 production of the $FcRI^+$ MØ population was abolished. Indomethacin also partially abrogated the MDP mediated suppression of $FcRI^-$ MØ PA activity. We will be examining the possibility that some other prostacyclin besides PGE_2 (such as leukotriene B_4), is mediating the suppression.

In summary, this has been a highly productive year. We have established several new methods to monitor immunosuppression developed in severely injured patients and have demonstrated that immunosuppressed patients have elevated MØ PGE_2 and MØ TNF production in conjunction to depressed MØ PA activity and poor clinical outcomes.

Methods

The Plasminogen Activator (PA) assay is performed as previously described: (1) The procoagulant assay and the lysozyme assay are also performed as previously described (1-2). The assay for suppression of MØ PA activity is performed by isolating the MØ as described (1), then recombining either 2 x 10^6 normal or 2 x 10^6 patient MØ with equal numbers (2 x 10^6) of the patients E-rossetted T cells. The mixed populations are co-cultured for 48 hours in Iscove's supplemented with 8% FCS and media supplements as described (1). In most assays, the normal and patient MØ are collected at day 1-10 post injury and cultured in supplemented Iscove's for 3-4 days. The patient T cells collected at 3-5 (early) or 13-22 (late) days post injury are co-cultured with the normal or patient MØ collected 3-4 days earlier.

MØ PGE_2 is measured with a newly developed enzyme-linked immunosorbant assay(ELISA) for PGE_2 detection in our laboratory this year. Microtiter 96 well plates (Nunc, VWR) were coated with 200μl/well goat anti-rabbit IgG in 5 x 10^{-2} M potassium phosphate buffer pH7.4 (Jackson Immuno Research Labs. Cat# 111-005-045) for 18 hours at room temperature. After extensive washing plates were saturated with enzyme immunoassay (EIA) buffer at 4°c for 18 hours. In the competitive binding assay PGE_2 standard (Sigma) or sample were added first followed by the acetylcholinesterase - PGE_2 (Enzyme tracer) conjugate (ACE™) (Commissariat a l'Energie Atomique, Institut de Recherche Fondamentale, Saclay, France), and rabbit anti-PGE_2 antibody (Sigma, P3038) for 18 hours at room temperature. Bound acetylcholinesterase activity was measured by adding enzyme substrate (Ellman's reagent) (Sigma) to the wells. Optical density of each well was detected at 410 nm on a Dynatech MR600 plate reader. Calibration curve was created from the dilution of PGE_2 standard and the appropriate % of fraction bound (B/B %)B/B_0 % was calculated by the following formula using our compaq computer which is connected with the densitometer. B/B % = (ST or SMP - NSB)/(B_0 - NSB) x 100, where NSB represents the nonspecific binding, B_0 is the mean absorbance of wells which have reached the maximum density, ST is the mean of each standard and SMP is the mean of each sample.

Determination of MØ tumor necrosis factor (TNF) production is performed by isolating the MØ as described (#1), then MØ are prestimulated with 10-100 μg/10^6 cells of human recombinant interferon-gamma (IFN_γ) (obtained from Collaborative Res. Inc.) followed by a second 20μg/ml MDP stimulation, 5-6 hours later. In the patients' assays, nor-MDP (a generous gift from Ciba-Geigy, Basal, Switzerland), an MDP derivative was used, which has greater activity inducing cytoxicity of MØ than MDP itself. Cells are cultured in Iscove's media supplemented with 15% FBS, antibiotics, fungizone and L-glutamine at 37°C. After 20 or 40 hours, culture cell-free supernatants are collected and kept at -80°C until further use. TNF and PGE_2 levels are determined in the supernates. Cells are deattached by EDTA treatment and scraping and kept frozen. After repeating freeze-thawing followed

by sonication, intracellular TNF and procoagulant activity (PCA) are determined.

Tumor necrosis factor was assayed using serum-free in vitro bioassay described by Kramer and Carver (#2) with slight modifications. Murine L-M connective tissue cells, a derivative of L929, were obtained from ATCC (CCL1.2) and grown in medium 199 supplemented with 0.5% Bacto-Peptone (M199/0.5% Peptone) (Difcon-laboratories, Detroit,MI), 1.35mg/ml sodium bicarbonate and antibiotics. Cell monolayers were detached with 0.25% trypsin treatment (GIBCO) extensively washed and subcultured every 3-5 days using 10^5 cell/ml density. A 96 well flat bottom microtiter plate (Costar) was seeded with $3x10^4$ cells/well in a 100μl volume of M199/0.5% Peptone, leaving well A1 cell free. Following a 22-24 hour incubation at 37°C in a specialty gas mixture (10% CO_2, 7% O_2, 83% N_2), medium was removed and replaced with fresh M199/0.5% Peptone. 100μl of TNF sample, standard or control was added to wells 1-12 of row A in duplicate. Serial two-fold dilutions were prepared from row A through row H, and 100μl volume from row H was discarded. Finally, 100μl medium containing 2μ/ml Actinomycin-D (Sigma, A-1410) was added to all wells. Plates were incubated 20-22 hours at 39°C. Plates were stained with 0.5% crystal violet (0.5g crystal violet in 20% methanol) (Sigma, C-6158). Excess stain was removed with repeated washing in tap water. Absorbance was determined at 570nm using a Dynatech MR600 plate reader.

Titers were expressed as the reciprocal of the dilution resulting in 50% cytotoxicity determined from the mean absorbance of 11 control wells in each plate. The endpoint corresponding to the 50% cytotoxicity absorbance value was used to calculate sample activity using the following formula:

$$\text{Sample Act (pg/ml)} = \text{Std Act (pg/ml)} X^{(\text{Sample EP} - \text{STD EP})}$$

MØ subsets are separated based on the density of high affinity Fc receptors for IgG2a, by rosetting MØ with anti-Rh coated human erythrocytes (Zembala et al) MØ subsets are stimulated the same was as that was described for the whole MØ population. Subset experiments are performed on trauma and burn patients to delineate the differential stimulation requirements and monokine production capacity of MØ subsets. We used cyclo-oxygenase inhibitor indomethacin at a concentration of $1x10^{-6}$M to block the MDP induced PGE_2 production of normal and patient MØ and MØ subsets.

[1] Miller, et al, 1982, J. Immun.,128:2194

[2] Kramer and Carver, 1986, J. Immun., 93:201

Results and Discussion

In the period covered by the second year of contract No. DAMD17-86-C-6091, several goals have been attained. Twenty-six patients have been nominated this year, including 14 trauma and 12 burn patients.

Of these patients four were studied in the first four months, eleven the next five months and eleven the last four months of the year. Of these patients two burn patients and four trauma patients have succumbed to fatal sepsis.

As illustrated in Table 1, we found reduced MØ functions in the burn and trauma patients who experienced immunodepression. As we have previously shown, there is a correlation between MØ PA depression and depression of phytohemagglutinin (PHA) induced T cell proliferation concomitant with elevation of MØ prostaglandin E_2 (PGE_2) levels and poor prognosis. As can be seen, there is a change in MØ PGE_2 and depression of MØ PA in some trauma as well as burn patients who experience no septic complications.

Experiments reported last year indicated an increased MØ PGE_2 production as a result of exposure of patient or normal MØ to T suppressor cells (T_s) collected from immunosuppressed patients as detailed in the methods. At that time we were hypothesizing that immunosuppression experienced by trauma and burn patients could be related primarily to elevated MØ PGE_2 levels. These increased MØ PGE_2 resulted from a shift in FcRI positive and negative MØ subsets towards the PGE_2 producer, Fc^+ subset. The other possible reason for the increased MØ PGE_2 in these immunoincompetent patients is the appearance of a non-specific T_s which increased MØ PGE_2 production. We have confirmed that the late appearing non-specific T_s which is $CD8^+$ does increase MØ PGE_2 production (Table II). However, we have determined that a $CD4^+$ T_s, arising early in the post-injury period, suppress MØ function but does not increase MØ PGE_2 production (Table II). This early T_s appears to have a Fc^- MØ as its target.

We have similar data showing the MØ induction by muramyl dipeptide (MDP) in patients and normals can suppress MØ functions by both increasing PGE_2 and by a non-PGE_2 mediated mechanism (Table 3). We have examined the stimulation capacity of MDP on patient MØ and MØ subsets to detect early post-trauma change in MØ responsiveness to bacterial stimuli. MDP is a potent PGE_2 secretagogue and we, and others have found that it also depresses MØ PA activity. In our last annual report we have already suggested the importance of a shift in MØ subsets in the post-injury immunosuppression. We examined the

subset ratios of patients MØ populations by separating out the MØ subset rosetting with the high affinity receptor for IgG2a/FcRI$^+$). This FcRI$^+$ subset has been previously suggested to contain the inhibitory MØ population. As we reported in our recently published paper (SIS, March), immunosuppressed patients had a dramatic increase in the proportion of their FcRI$^+$ MØ subset which coincided with an increase in their PGE_2 activity. In our recent experiments done in the last quarter of this year we used indomethacin in vitro to block MDP induced MØ PgE_2 production by patient MØ. As can be seen in table 3, a cyclo-oxygenase inhibitor, indomethacin reverses MDP inhibition of MØ PA responses in both FcRI positive and negative subsets, suggesting that FcRI$^-$ MØ PA depression of MDP is possibly mediated by another cyclo-oxigenase product (eg. LTB_4) and not by PGE_2, or by some other factor(s). However in the FcRI$^+$ MØ population, MDP induced PA depression was reversed by indomethacin to the normal level. These preliminary data coming from experiments done on two patients need to be confirmed in the next quarter. Since ibuprofen therapy is now being used in clinical trials in trauma and burn patients, we need to understand the possible advantages and disadvantages in its action.

We made a progress this year in testing MØ functions of post-injury patients developing a bioassay for determination of tumor necrosis factor (TNF). TNF-alpha, also called cachectin, is a monokine produced by stimulated monocytes, having a major role in the metobolic development in cachexia and septic shock. Therefore we examined the TNF production of patients and normal monocytes and MØ subsets after different stimuli. As shown in Table 4, stimulation of patients or normal MØ with MDP or IFNγ alone could not induce TNF production. However, combination of stimuli, IFNγ plus MDP induced both patients and normal MØ TNF production. In developing the TNF assay for patient populations, we found that maximal stimulation of TNF with 20μg/ml of MDP and 100U/ml of IFNγ often obscured differences in response between patients and normals. In contrast, suboptimal stimulation with INFγ (10U/ml) + MDP resulted in a large response from immunosuppressed patient MØ while only a minimal response was stimulated in the normal population. Using this protocol we have also demonstrated that the FcRI$^+$ MØ population in both controls and immunocompromised patients produces the majority of TNF in response to stimulation by bacterial cell wall products and IFNγ (Table 5) This is a significant new finding and important because of the shift toward FcRI$^+$ MØ population seen in immunocompromised patients. The post trauma in vivo environment contains both bacterial cell wall products and activated by lymphokines.

These stimuli would induce increased TNF production in patients with a shifted MØ FcRI$^+$ subset ratio and could cause cachexia and metabolic derangement. Although Dr. Faist (personal communications) has indicated that elevated TNF levels occur in his patients, no published data demonstrating elevated MØ TNF in immunocompromised trauma patients have appeared in the literature. Our data (Table 4) clearly shows an increase in immunosuppressed patients' TNF levels. These patients also have increased leukocyte pyrogen activity which may be the result of their elevated MØ TNF production. We interpret this

data as implying that the increased TNF production by the immunosuppressed patients' MØ population may be accounted for by the presence of greater numbers of Fc^+ MØ.

Preliminary experiments are also underway examing the effect of blocking cyclo-oxygenase products in these patients. Since MØ PGE_2 production is a major contributor to post trauma immunosuppression, it is reasonable to consider blocking the synthesis of this cyclo-oxygenase product by administering ibuprofen to patients. In vitro blocking of MØ PGE_2 production with indomethacin resulted in an unexpected effect on MØ TNF production. We are examining both secreted and intracellular TNF and finding that normal MØ respond by secreting most of their TNF (TABLE 5). Intracellular TNF also rises in the normal MØ after stimulation but not to levels above those secreted. In contrast, in studies on two immunosuppressed patients' MØ we found massively increased intracellular TNF levels as well as increased secreted TNF. Addition of indomethacin to these patients' MØ actually resulted in increasing their MØ TNF release. If these results are consistently found in further studies, it may present a problem for clinical administration of cyclo-oxygenase inhibitors to immunosuppressed trauma patients. Increased TNF release with consequent endotoxin shock might result from such ibuprofen therapy. With the aid of Dr. Thomas Takayama, we are moving forward with the thermally injured guinea pig model. We will test MØ TNF production after thermal injury in this model and examine the in vivo effect of administering ibuprofen to these animals. At a recent meeting it was reported that endotoxin shock was occuring in some patients receiving ibuprofen. This information makes testing both the patient MØ TNF response after ibuprofen and the guinea pig MØ response critical to evaluating clinical trials. The shift in MØ subsets we have identified have several implications. The patients exhibiting such a MØ subset shift will experience increased TNF levels, increased PGE_2 levels, depressed Antigen presenting function and depressed PA activity. The ability to identify these shifts in patients MØ populations should signifi antly simplify the monitoring of trauma patients immune profile and be directly applicable to identification of combat casualties at risk of infectious complications. In addition, development of a sensitive assay for patient MØ TNF levels will improve ability to detect the onset of endotoxin shock in trauma victims.

Table 1

CORRELATION OF MØ PA DEPRESSION, PGE_2 AUGMENTATION AND CLINICAL OUTCOME

Pt.	Injury	ΔPHA%[a]	PA (01-13)[b]	ΔPGE_2 ng[c]	Outcome
1. Do	burn	-42	53.7→39.7	4.2→48.5	No Complications
2. Ho	burn	-28	39.0→11.2	3.5→54.6	Multiple Septic Episodes
3. Sh	burn	-37	41.7→31.3	12.5→20.2	No Complications
4. Lo	burn	-43	31.3→28.1	3.9→23.5	No Complications
5. To	burn	-28	32.0→17.2	6.9→42.0	Multiple Septic Episodes
6. Cr	burn	-80	54.8→17.0	9.5→15.9	No Complications
7. Al	burn	-66(11)	30.2→9.1	ND.[d]	Fatal Sepsis
8. Cr	burn	-74(12)	33.9→16.8	13.2→32.0	Multiple Septic Episodes
9. Ho	burn	-75(8)	35.7→14.9	54.0(15.0)	Multiple Septic Episodes
10. Sm	burn	-90(7)	19.6→7.7	6.2→10.0	Fatal Sepsis
11. Al	trauma	-63	31.4→24.4	8.3→20.3	No Complications
12. Er	trauma	-49	50.1→32.3	6.2→24.5	No Complications
13. Ca	trauma	-75	53.5→23.2	10.5→44.3	Died
14. Sc	trauma	-14	26.3→20.7	11.0→36.0	No Complications

15.	Fo	trauma	-84	38.8→18.3	4.1→29.0	Died
16.	Tu	trauma	-82	37.5→11.0	6.2→51.0	Infectious Episode
17.	Bu	trauma	-76(8)	30.8→8.8	→46.00	Septic
18.	Ch	trauma	-66*	28.3(27.0)	N.D.	Fatal Sepsis
19.	Je	trauma	-47(4)	16.8→10.3	34.0→37.00	Septic Complication
20.	Yo	trauma	-91(5)	37.1→10.6	3.6→16.00	Multiple Septic Episodes

a) Maximum change at 2-12 days post injury in mitogen induced proliferation of $2x10^5$ peripheral blood mononuclear cells to 2μg PHA.

b) Maximum change in MØ plasminogen activator (PA) activity from initial response to most depressed within the first 13 days post injury. PA activity measured in % plasmin specific fibrinolysis.

c) Maximum protaglandin E_2 production in first 13 post injury days measured as nanograms per 10^6 recovered MØ.

d) Not done.

Table 2

COMPARISON OF T_s ACTIVITY OF EARLY AND LATE T CELLS FROM IMMUNOSUPPRESSED PATIENTS[a]

T Cell Source	PA(% fibrinolysis)[b]		PGE_2 pg/10^6		MØ($x10^{-3}$)[c]	
MØ Source	Days Post Injury[d]		MØ	MØ+T_s	MØ	MØ+T_s
Norm	22		43.3 → 25.7		6.2 → 17.1	
Pt	22		27.8 → 20.1		13.1 → 19.9	
Norm	21		40.9 → 17.7		8.4 → 24.3	
Pt	21		26.0 → 20.6		20.6 → 28.0	
Norm	16		34.5 → 16.5		11.3 → 17.4	
Pt	13		28.7 → 13.4		14.6 → 30.5	
Norm	5		56.2 → 38.4		1.7 → 1.7	
Pt	5		29.8 → 22.2		2.6 → 3.9	
Norm	3		50.0 → 29.4		3.6 → 3.5	
Pt	3		36.4 → 26.3		4.0 → 4.9	

a. EA-rosetted, MØ depleted T cells collected from patients with depressed MØ PA activity (immunosuppressed) were co-cultured with equal numbers of autologus patients or allogeneic normal MØ for 48 hours. (see Methods)

b. PA responses of MØ cultured alone or MØ co-cultured with patient T cells.

c. PGE_2 responses of MØ cultured alone or MØ co-cultured with patient T cells.

d. Day of T cell collection either late (>12 days) or early (>10 days). T cells recombined with MØ collected 2-3 days earlier.

Table 3

NON PGE_2 MEDIATED MDP INHIBITION OF MØ SUBSET PA RESPONSES

MØ SOURCE[1]	PA (% fibrinolysis)	PGE_2 ng/10^6 MØ
Total	34.4±1.6	32±12
Total + MDP[2]	25.0±2.1	72±23
$FcR1^+$	26.0±5.4	31±8
$FcR1^+$ + MDP	14.6±3.1	76±24
$FcR1^-$	39.0±6.7	4.0±0.8
$FcR1^-$ +MDP	29.2±7.4	5.5±1.9

Indomethacin reverses MDP inhibition of MØ PA responses in patient:

MØ Source	PA (% fibrinolysis)
$FcR1^+$ + MDP	11.3
$FcR1^+$ + MDP + Indo[3]	20.2
$FcR1^-$ + MDP	10.6
$FcR1^-$ + MDP + Indo	25.2

1. Unseparated MØ, or MØ rosetted with α Rh coated human erythrocytes = $FcR1^+$ MØ, non-rosetting = $FcR1^-$
2. MØ stimulated with 20μg/ml MDP
3. MØ cultures contain 20μg/ml MDP and 10^{-6} M Indomethacin

Table 4

INCREASED TNF[1] PRODUCTION BY IMMUNOSUPPRESSED PATIENTS[2]

MØ Source	Day Post-Injury	Stimulated with: MDP[3]	IFNγ[4]	IFNγ + MDP[5]
ptY	27	0	0	3582
norm		0	0	459
ptH	15	0	0	1679
norm		0	0	0
ptS	16	0	0	2015
norm		0	0	0
norm max. stim.[6]:				$\bar{x}$ 2643±1395

1. Tumor necrosis factor measured as pg produced by 10^6 MØ.
2. Immunosuppressed patients defined as having depressed MØ PA and PHA responses.
3. MDP was used at 20μg/ml concentration (suboptimal level) to stimulate MØ for 20 hours.
4. 10μ/10^6 MØ IFNγ was the stimuli for MØ.
5. Combination of 10u 1FNγ/10^6 MØ with 20μg/ml was used for stimulation of MØ TNF.
6. Maximal stimulatory dose: 100u of IFNγ/10^6 MØ plus 20μg/ml MDP.

Table 5

INCREASED TNF PRODUCTION BY PT MØ/Fc^+ SUBSET

	Stimuli		
	None	5UIFN$_\gamma$ + 20μgMDP	100U IFN$_\gamma$ + 20μgMDP
NORMAL	398	993	1808
PT	1218	2170	6706
FC^+ NORMAL	104	1396	8708
PT	1638	4825	20268
Fc^- NORMAL	0	608	1029
PT	0	392	2779

Table 6

TNF PRODUCTION BY MØ SUBSETS AT 20 HOURS
TNF pg/10^6 MØ

	Secreted	Intracellular	Total
MØ	0 ±0	0 +0	0
MØ+IFNγ+MDP[1]	2643 ±1395	1992 ±1233	4635
MØ+IFNγ+I1-2[2]	1399 ±810	1729 ±1588	3227
$FcR1^+$	243 ±387	572 ±895	815
$FcR1^+$+IFNγ+MDP	6995 ±4023	3218 ±997	10213
$FcR1^+$+IFNγ+I1-2	2854 +1733	2991 ±1608	5844
$FcR1^-$	21 ±51	225 ±558	243
$FcR1^-$+IFNγ+MDP	3960 ±3989	1407 ±928	5366
$FcR1^-$+IFNγ+I1-2	1813 ±1533	1961 ±1500	3744

1. MØ were stimulated with 100U/10^6 cells of IFNγ plus 20μg/ml MDP
2. MØ were stimulated with 100U/10^6 cells of IFNγ plus 1000U/10^6 cells of I1-2

DISTRIBUTION LIST

4 copies	Commander Letterman Army Institute of Research (LAIR), Bldg. 1110 ATTN: SGRD-ULZ-RC Presidio of San Francisco, CA 94129-681
1 copy	Commander US Army Medical Research and Developmen Command ATTN: SGRD-RMI-S Fort Detrick Frederick, MD 21701-5012
2 copies	Defense Technical Information Center (D ATTN: DTIC-DDAC Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Servies University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234-6100