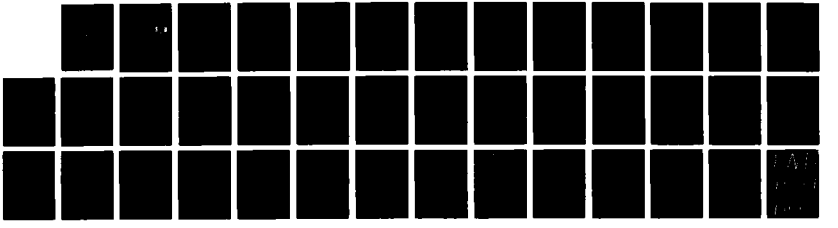
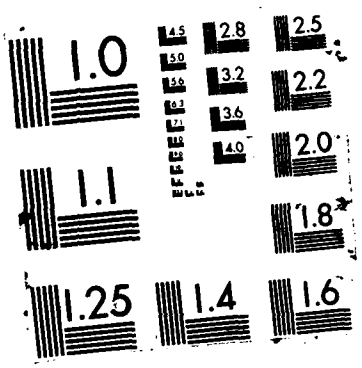
AD-A185 460 STUDIES OF ALTERED RESPONSES TO INFECTION INDUCED BY THERMAL INJURY(U) CALIFORNIA UNIV SAN FRANCISCO C L MILLER 20 JUL 87 DAMD17-82-C-2001 1/1

UNCLASSIFIED F/G 6/5 NL




1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3
7.1
8.0

12

AD _____

STUDIES OF ALTERED RESPONSES TO INFECTION INDUCED BY THERMAL INJURY

FINAL REPORT

Carol L. Miller, Ph.D.

July 28, 1987

January 1977 - April 1986

Supported by

U.S. Army Medical Research and Development Command

Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD 17-82-C-2081
and
Contract No. DAMD 17-77-C-7012

University of California
San Francisco, California 94143

A185 460

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: Univ. of California; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): 145 Irving Street, San Francisco, CA 94143	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: US Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD 17-82-C-2081, DAMD 17-77-C-7012
8c. ADDRESS (City, State, and ZIP Code): Ft Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62772A	3S1-62772A 874	AD	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
Final	FROM Jan. 77 TO Apr. 86	87/07/28	38

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		Trauma, Monocyte dysfunction, immmunosuppression
06	05		

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

Over the life of this contract, we have made the initial demonstration of the post trauma generation of T suppressor lymphocytes, we have determined that nutritional depletion is not the trigger but rather the result of post injury cellular abberations, we have adapted and developed number of new assays for assessing trauma patients cellular immune function which include: a measurement of MØ Plasminogen activator production, an assay for MØ procoagulant activity, an assay for patient T suppressor cells which uses a MØ target and requires a 2 day incubation rather than a 6 day incubation, and an assay for patient leukocyte pyrogen production. Using these assays, we have defined a number of crucial defects in both monocyte and T cell functions which are correlated with post trauma immunosuppression. Our current hypothesis is that alterations in crucial MØ T cell interactions may be the underlying mechanism of post trauma immuno-suppression. In addition, we have examined several types of prophylactic therapies which are directed toward modulating the monocyte and T cell defects we described. Our data seems to indicate that a combination of defects may be resulting in the immunocompromised trauma patient syndrome. Effective immunotherapy may require a combination of immunotherapeutic)(OVER)

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 M. Miller	301-663-7325	SGRD-RMI-S

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

19. Abstract

(cont'd)

agents to contravene these multiple defects. The assays which we have developed are therefore important in the monitoring of the patients for the efficacy of immunotherapy as well as for detecting post trauma alterations in patient leukocyte function.

Keywords; coagulation

Abstract pg ii

Accesion For	
NTIS CRA&I	☑
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and / or Special
A-1	

FOREWORD

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

In conducting research using animals, 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 (NIH Publication No. 86-23, Revised 1985).

TABLE OF CONTENTS

	Page Nos.
Report Documentation Page	ii
Foreword	iii
Introduction	1
Results and Discussion	5
References	19
List of Publications	24
Distribution List	34

TABLES

Table 1 - Representative Burn Patient Immune Profile	27, 28
Table 2 - Range of C Synthesis Response by Normal MØ After Stimulus with Fc	29
Table 3 - Range of C synthetic Response by Normal MØ After Stimulation with PHA Induced Lymphokines	29
Table 4 - Failure of PPD to Stimulate Normal Individual's MØ C Production	29
Table 5 - Decreased C2 Synthesis by Burn Patient MØ Collected at Various Days Post-Burn	30
Table 6 - Correlation of PGE_2 with Massive Increase of PGE_2 at 1-4 Days Post-Burn	30
Table 7 - Elevation of MØ LP Production Concomitant to Depressed MØ Immune Function and Unaltered MØ PCA Activity	30
Table 8 - Suppression of MØ PA Production by Con A Activated Cells PA as % Fibrinolysis	31
Table 9 - Suppression of MØ PA Concomitant to Enhanced LP Production	31
Table 10 - Effect of Patient T Cells on MØ Function	32
Table 11 - MØ PA Fibrinolytic Units Produced by 5×10^5 MØ Cultures	33

Introduction

The severely injured patient develops host defense defects which can be directly correlated to an increased incidence of septic complications. In particular, development of augmented regulatory cells, both inhibitory monocytes (inh MØ) and suppressor T lymphocytes (T_s), have been linked to the post-trauma immunoincompetence. The hypothesis under investigation in this laboratory is that inimical alterations in vital monocyte-T cell interactions play a pivotal role in the development of excessive regulatory cells and the depression of the specific immune systems which occur post-trauma. In addition, we postulate that these MØ dysfunctions are important contributors to the depression of other non-specific host defense systems. Two MØ functions, antigen presentation in association with immune response antigens (Ia) and mediator production (Interleukin-1), are absolutely required for initiation of antigen specific responses by T helper lymphocytes (Th). An interruption to either of these crucial functions leads to decreased immune function and augmentation of T_s development. It is our contention that changes in certain MØ functions mirror alterations in MØ immune capacity and differentiation status. Trauma patients have been demonstrated to have depressed immune capacity and excess immune regulatory activity. Consequently, we contend that monitoring certain crucial patient MØ functions not only allows characterization of patients' host defense status, but also promotes understanding of mechanisms which control the balance between immune stimulation and immune regulation.

In order to detect aberrant MØ-T cell interactions, purified and isolated cell populations (e.g. fac MØ plus T_h, free of inh MØ or T_s) must be employed.

When we initiated our trauma-patient studies, no such purification techniques were available and no T_s activity had been detected before 7 days post-injury. 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. In any assayed system which has T cells and MØ, there is a confusion in data interpretation between MØ mediated defects and 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, 2). We demonstrated that depressed MØ PA function appeared several post-trauma days before detectable T_s and that PA depression correlated both to mitogen hyporesponsiveness and increased septic complications (3, 4). These data indicated 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 (5). However, 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 all monokine activation of T-cell subsets since it assesses thymocyte proliferation. Therefore this assay is not specific for IL-1. The LAF assay would fail to detect a shift in monokines that activated T_h (IL-1) to that which activated T_s. Both PGE_2 and other inhibitory monokines are known to preferentially increase T_s proliferation (6-8). Several laboratories, including our own, have shown an increased PGE_2 production occurring almost immediately post-trauma (9, 10). Therefore, the detection of unaltered LAF activity post-trauma fails to discriminate between normal fac MØ function and 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, 12). Our data indicate increased MØ LP activity in trauma patients who develop septic complications.

Other MØ secretory products like complement (C) components and procoagulant activity (PCA) also have potent effects on the immune system (13-15). Synthesis of these MØ products is also altered post-injury (16, 17). C synthesis decreases while PCA generation increases (16-18). 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.

Recent development of monoclonal antibodies and the availability of sorting techniques have made isolation of T cell and MØ subsets feasible. Anti-T8 monoclonal antibodies distinguishes the $T8^+$ expressing human T suppressor/cytotoxic cells (T_s) from those human T helper/inducer (T_h) cells, which express T4 and can be detected with anti-T4 monoclonal antibodies. The $T4^+$ T helper/inducer cells can be further divided into suppressor inducers (T_s inducer) and helper inducers (T_h inducer). The T_s inducers can be isolated by using another group of monoclonals (anti-leu 8 or anti-2H4 monoclonal antibodies). Once the $2H4^+$ T_s inducers have been removed by panning techniques, only T_h inducers should remain in the $T4^+$ population. $T4^+$ $2H4^-$ cells both act as helper inducer (i.e., interact with APC) and produce IL-2.

MØ complement receptors (CR) and Fc receptors may also identify stable differentiation subsets. Only about 50% of MØ can be induced by lympokines to express CR receptors (19). Lymphokines seem to drive MØ differentiation in a one-way fashion (20, 21). However, most lymphokines act on Fc and CR bearing cells, not by changing the number of receptors expressed but, by enhancing the activity of that particular MØ subset by some other mechanism (21, 22). Once expressed, CR3 or C3bi(OKM1) and Fc numbers are not changed by adjuvant stimulation (20, 21, 22, 23). In fact, the ability of MØ subsets to respond to

certain inflammatory stimuli depends on the expression of these markers (CR1, CR3, Fc) (21, 22).

Post-trauma depression of monokine activity could reflect a change in the MØ 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 (24, 25). The appearance of such a T_s subset after trauma would severely compromise fac 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 (1) it was genetically restricted, (2) it had only a MØ APC target, and/or (3) its effect was solely detectable on target cells with increased susceptibility to suppression (patient's own cells) (26, 27,28, 29). Most of the current assays for trauma patients' T_s assess suppression of third party allogeneic MLR or of mitogen responses (30, 31). Neither the MLR or the mitogen assay detect genetically restricted T_s. The mitogen assay would fail to detect a T_s with an APC MØ for a target. The MLR would obviously not detect a genetically restricted T_s. Based on murine data characterizing MØ-T_s interactions, we concluded that the appearance of a genetically restricted T_s immediately post-trauma is probably not the initial trigger of immune unbalancing. Nevertheless, we have concentrated on developing an assay with patient cells which detects suppression of the patients own MØ. This system would detect a genetically restricted T_s.

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 cells interactions may not reflect MØ activity at local sites. Therefore, it is 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.

If it can be shown that the alterations detected in subset interactions are due to stable physiological changes, it should be possible to develop a phenotype profile of the aberrant MØ. Since Fc, CR and Ia expression appear most effected by MØ differentiation state, they might be markers which are altered in MØ subsets whose MØ-T interactions are aberrant. A stable marker phenotype could be established for aberrant MØ and linked to their altered immune functions. Once this is accomplished, a one-day monitoring of trauma patient immune deficiencies could be performed by fluorescent cytometric analysis to quickly identify the patient at risk. In addition, prophylactic therapies specifically designed to correct the MØ-T cell pathway imbalance could be initiated. An excess of T_s and depressed MØ Ag presenting capacity due to decreased Ia expression could be treated by expanding the T_h population (TP5) and increasing MØ Ia expression (IL-2) (32, 33). An excess of PGE_2 producing MØ could be treated with administration of prostacyclase inhibitors. Finally, the availability of a phenotypic profile should facilitate monitoring of the corrective effects of prophylactic therapy on the defective cells.

Results and Discussion

During the life of this contract, 271 patients have been studied of which 106 were burn patients and 65 were trauma patients with an ISS of >20. Of these patients, 35 expired from septic complications. A representative group of 26 burn patients is presented on Table 1. In our original army supported work, we utilized a murine model to establish that there was a defect in the overall

immune response after thermal injury (Publication #1). In that original work performed in the murine model, we demonstrated that B lymphocytes were not defective but that the burn induced immune defect was at the level of the T cells and/or MØ. At the same time, we were examining T lymphocyte proliferation in patient peripheral blood mononuclear cells to determine if a similar type of T cell defect was also present in patient populations (Publication #2). We found that trauma patients not only had reduced T lymphocyte proliferative function but that T suppressor lymphocytes were generated as a result of both thermal injury and severe trauma (Publications #3-6). This was the first demonstration of the actual generation of T_s lymphocytes in patient populations although their existence had been postulated by Munster (34).

One mechanism by which T lymphocyte defects could be generated and excessive T_s activity could be induced revolved around MØ defects. The immunological literature of that period was just beginning to examine the role of MØ in T lymphocyte activation. A few papers appeared which suggested that depletion of antigen presenting MØ resulted in increased generation of T_s (35). Alternatively, other authors suggested that increased MØ PGE_2 activity would increase T_s activity (36). This laboratory began to examine the monocyte functions of trauma patients to determine if MØ dysfunctions could be correlated with immunoincompetence. In a series of papers, the laboratory explored a number of different monocyte functions to determine if patient MØ function was altered. Initially, we chose to assess monocyte production of plasminogen activator (PA), monocyte procoagulant activity (PCA) or tissue factor (TF), and MØ lysozyme production. We selected these activities because MØ PA production had been suggested to be correlated to antigen presenting function while excessive coagulation (and possibly MØ PCA) had been linked to increased septic episodes (Publications #7-13). MØ lysozyme production had been demonstrated to be relatively unaffected by immune stimuli and to serve as a MØ viability marker. Not only was this laboratory one of the first to describe post trauma

MØ dysfunction, but we also developed and adapted for patient use a number of MØ assays. We are still one of the few laboratories monitoring MØ plasminogen activator function. In a series of publications over the next several years, we demonstrated that an inhibitory MØ (one possibly producing PGE_2) was induced in the trauma patient population. It was clear both from our own and others' work that MØ could interact with T cells in two ways. MØ could turn on or facilitate T cell function or MØ could inhibit T cell function. Patients appeared to have an increase in inhibitory MØ (Publications #7-15).

Correlation of patient MØ and T cell aberrations with the patients' clinical course allowed us to define the immune defects in the trauma patients which were predictive of the development of immunoincompetence. We then began to investigate mechanisms by which trauma might mediate these defects (Publications #19-27). Several possible mechanisms were suggested by data in the literature. One possibility was that the metabolic derangement that follows trauma was leading to protein depletion. Protein depletion was suggested as leading to immune dysfunction by not allowing the protein synthetic process necessary to produce lymphokines and/or antibody. Consequently, we examined the effect of nutritional deprivation on immune functions (Publications #16-18) and the ability of nutritional support to ameliorate immune dysfunctions. We found that the ability of B lymphocytes to produce specific antibody in response to antigen and T lymphokines was conserved in protein depleted animals up to the point of almost total protein deprivation. A number of other protein dependent systems were affected before B lymphocyte function. In another set of studies, we examined the ability of nutritional support to prevent the development of MØ defects in trauma patients. What became apparent was that the post trauma development of excessive protein catabolism was associated with, but was not necessarily responsible for, the development of MØ dysfunction. Nevertheless, nutritionally supporting the patients with intravenous solutions of amino acid plus glucose did not prevent the development of MØ dysfunction but did reduce

some of the cachexia experience by the patients (Publication #18). At that period, it was not known that the monocyte products or monokines such as tumor necrosis factor (TNF) and Interleukin-1 (IL-1) induced cachexia and proteolysis (Publications #37, 38). We concluded that some connection existed between MØ dysfunction and post trauma protein depletion but that protein depletion was not causing the immune dysfunction. In our current studies, we are actually measuring patient MØ TNF and IL-1 production post trauma. However, even early in our studies we reported a link between metabolic derangement and MØ dysfunction.

In order to determine how trauma could result in an effect on cells of the distal immune system, we next concentrated on the effect of trauma on the production of inflammatory mediators that were known to affect immune cell function at a distance. For example, both fibrin degradation products and complement split products had been suggested as depressing T lymphocyte function. The cellular target for these inflammatory mediators was undefined, but it was known that MØ had both fibrin receptors and complement receptors whereas T lymphocytes appeared to have no fibrin receptors and few complement receptors. The target for complement and fibrin was therefore most likely a MØ. Adequate monocyte function was known to be necessary for T cell secretion of lymphokines and proliferation. In addition, trauma produced massive elevation of both fibrin degradation products and complement split products. Because of this association between MØ with receptors for fibrin and complement and trauma induced products, we assumed that trauma might ultimately affect immune function by its effect on MØ production of inflammatory products. Therefore, we examined the production of patient MØ Procoagulant Activity (PCA) or tissue factor which had been previously suggested to be involved in fibrin deposition. We examined post trauma MØ production of PCA and found that it was elevated in all trauma patients and significantly elevated in patients who developed thromboembolic complications (Publication #22). These data implied that there might be an

increase in immunosuppressive fibrin degradation products secondary to MØ mediated increased fibin deposition. However, the increase in MØ PCA levels did not seem to be as evident in thermally injured patients and was less germaine to their development of immunosuppression. The importance of elevated MØ PCA levels in trauma patients splenectomized as the result of their injuries was more apparent (Publications #22, 23).

To further study the possible association between post trauma elevation in complement (C) split products and immunosuppression, we also examined MØ production of complement component C2. Initial experiments were designed to assess our ability to utilize the complement plaque assay (Publication #31). C components are monokines which may be influencing not only immune function but also neutrophile function. Many C split products are inhibitory to MØ and T_h function while others increase MØ activation (13-15, 39-46). Complement split products are also crucially important in neutrophile chemotaxis and phagocytosis. MØ synthesis of some of the C components (C4, C3, C2, C5, Factor B) controls their concentrations at the local injury site (47-52). Consequently, a decrease in MØ synthesis of various critical C components could lead to insufficient C levels at the injury site even though no decrease in serum complement levels was detected. We have monitored the level of C2 synthesized spontaneously and after <u>in vitro</u> stimulus of the MØ Fc receptor by immunoglobulin fragments as was previously demonstrated. Data in Tables 1, 2, 3, and 4 illustrate results from our complement experiments. We first established that the crystalline fragment of immunoglobulin (Fc fragment) produced the best stimulation of C2 synthesis (Table 2). PHA-induced T-cell lymphokines stimulated synthesis more variably than did the Fc fragments (Table 3). To study other possible stimulators of MØ C synthesis, we assessed the effect of PPD on MØ. Data from the literature had suggested that PPD was a good stimulator of some monocyte functions. In our hands, PPD failed to

increase the levels of MØ C synthesis over that spontaneously seen in culture (Table 4). Consequently, we decided to use IgG Fc fragment as the stimulating agent. We also compared 2 days vs. 4 days of in vitro stimulus with the Fc fragment to ascertain where maximum C synthesis occurred. It appeared that most normal individuals showed tripling of C synthesizing MØ between unstimulated and stimulated cultures after only 2 days of incubation. After 4 days of culture the difference between stimulated and unstimulated MØ C synthesis was only two-fold. However, the maximum absolute number of detectable plaques (i.e. C synthesizing MØ) was greatest after 4 days of culture. Consequently, we have chosen to assay patient and normal responses after 4 days of culture to ensure that the maximum number of C synthesizing MØ is always detected. The assay is, therefore, weighed in the patient's favor and against detecting a defect. Even in this assay, however, it is quite apparent that there is a major and significant difference between MØ C synthesis by patients and by normals (Table 5). After severe injury, the MØ at the injury site should be activated to increase C synthesis. In fact, patient MØ are unable to respond to immune stimuli with increased MØ C synthesis. Consequently, our data suggest that an immunoincompetent trauma patient would not only have reduced C levels because of decreased lymphokine activity, but also that the MØ themselves would have reduced synthetic capacity. The level of fresh C available at the injury site for PMN chemotaxis and phagocytosis would be drastically reduced in these patients (Publications #31, 33). One of the problems of continuing the monitoring of the patients' C2 levels is that the assay requires a fairly large number of isolated patient monocytes. Although depression of C2 levels may be reflective of depression of synthesis of other vital complement components such as C3 and C5, the evidence for coordinate C synthesis has only been demonstrated for C2 and C4. Consequently, this assay may not be indicating a reduction in MØ C3 synthesis since it is C3 split products that are immunosuppressive. It is

not clear that measurement of patient C2 synthesis gives us the best return in information for the number of MØ needed. In addition, it has been demonstrated that a rise in MØ PGE_2 will dramatically depress MØ C synthesis. Consequently, the decrease in patient MØ C synthesis that we observe may be simply another indicator of increased patient MØ PGE_2 synthesis. This laboratory no longer is monitoring patient MØ C2 levels since measurement of MØ PGE_2 is probably more relevant and requires no extra patient MØ isolation.

The effect of elevated MØ produced PGE_2 was being widely described in immunology literature. In addition to depressing MØ complement synthesis, PGE_2 decreases MØ Plasminogen activator production, MØ Interleukin 1 production, T cell IL-2 production, MØ chemotaxis, and activates T_s. Any major elevation of MØ PGE_2 production would, therefore, have major depressive effects on patients' host defense systems. Elevation of MØ PGE_2 could result in many of the defects described in trauma victims including reduced MØ PA production, depressed T lymphocytes, mitogen responses, increased T_s activity, depressed IL-2 production, and decreased C levels. Major difficulties were being encountered, however, in assays for direct PGE_2 measurement. This laboratory initially used an RIA against PGB for the measurement of PGE_2 in the supernates from burn patients MØ. This initial commercially purchased assay was dependent upon the extraction of all prostaglandins other than PGE_2 from the samples and then the conversion of the PGE_2 to PGB. The PGB levels were then measured on an RIA with antibody to PGB. This assay was not only time consuming but it also had extremely low sensitivity. The extraction was variable, sometimes resulting in PGE_2 loss or less than adequate removal of other PG. The antisera was not monospecific and produced cross reactivity with contaminating PG of types other than PGE_2. When we added known amounts of PGE_2 to the media and fetal calf serum, we found that the detection level over the high background was about 10,000 pg/ml. Consequently, the pg amounts detected for the patients were not quantitative. Because of the above mentioned and other problems with the PGE_2

assay, there is little data in the literature regarding measured PGE_2 levels in trauma immunosuppression. Nevertheless, in assaying burn patient MØ samples we were able to detect much higher levels of PGE_2 than in normal control MØ samples. Although we had doubts about the quantitative values, it was obvious that a qualitative difference existed between normals and controls (Publications #27, 28). This first indication of massively increased PGE_2 in patient samples lead us to adopt another commercial assay system which utilized a monospecific anti-PGE_2 antisera and allowed direct detection of patient PGE_2 without conversion or extraction. Using this assay, we were able to obtain a good measure of the PGE_2 (Table 6). The primary difficulty with this assay is that it takes 2-3 days to complete and has an accuracy range of 300-15,000 pg. At higher PGE_2 concentrations, the accuracy falls off. Consequently, many samples needed to be run multiple times in order to get all the samples in that assay diluted to a concentration which puts them into the 300-15,000 range. In addition, the commercial tracer (I^{125}-PGE_2) is only prepared once every six weeks. As it decays, the accuracy range of the assay narrows even further. Consequently, we often had to repeatedly run our samples to obtain accurate quantitation of the PGE_2 amounts. Most recently, we have adopted an ELISA assay for PGE_2. This new commercial kit has much greater sensitivity, an overnight assay time, as well as automated data calculation. The Department of Surgery recently purchased a Dynatech plate reader. This reader is used for the PGE_2 ELISA, for an assay for tumor necrosis factor, as well as RIA. The software for data analysis which we use for all these assays is run on the Compac 286 purchased on this army contract. With this latest PGE_2 assay, we expect to be able to expand the number of patient samples we assess for PGE_2.

The elevation of PGE_2 is probably one of the most critical events in determining post trauma immunosuppression. Recently reported data indicates that the addition of exogenous IL-2 can not reverse the down regulator of T cell proliferation induced by PGE_2 (53). These data imply that although IL-2

secretion is depressed in immunosuppressed trauma patients, IL-2 therapy would be ineffectual in restoring immune function. Another potentially important MØ dysfunction in trauma patients is the metabolic derangement that is a feature of severe trauma. Excessive protein catabolism, cachexia, and overactivation of acute phase reactants are all characteristic of severe traumatic injury and have been attributed to the post traumatic release of a MØ product (37). One of the paradoxes of the immune suppressive trauma patient syndrome is that a split product of the monokine IL-1 is supposedly responsible for the aberrant metabolic function in these patients, yet MØ turn on of T cells which requires IL-1 is reduced in these patients. Although originally several investigators reported no decrease in MØ IL-1 after severe injury (5, 54), some of these same investigators and several other laboratories have seen depressed MØ IL-1 activity post trauma and sepsis (55). IL-1 can also act as a leukocyte pyrogen (56). Consequently, we examined the leucocyte pyrogen production in post trauma patients. We initiated a modification of the Bodel leukocyte pyrogen (LP) assay to measure the LP production of isolated patient MØ as can be seen in Table 7. In contrast to our expectations, patient MØ LP activity was markedly elevated in the face of depression of MØ PA production. We have confirmed this finding in a wide number of patients and have shown that increased LP activity coincides with septic episodes (Publication #36). It is possible, therefore, that the marked increase in MØ patient LP may be a result of septic episodes rather than a cause of immunosuppression. Nevertheless, we have also been able to show that T_s induce a marked increase in MØ production of LP while concommitantly depressing the MØ PA response. These data indicate that immunosuppressed patients who have excessive T_s would develop MØ with elevated LP activity.

Our original demonstration of the development of excessive T_s activity was done by showing suppression of normal T cell proliferation in a mixed lymphocyte reaction (MLR). However, in the past two years, we have been using a system in which we measure T_s ability to suppress MØ PA production. The motivation for

changing suppressor assay systems was that the MLR assay required large cell numbers and required 6 days of incubation. In addition, we were aware that this system would only allow detection of nonspecific genetically nonrestricted suppressor cells. In animal models thermal trauma results in the early generation of an antigen specific suppressor inducer cell which is genetically restricted (57). Consequently, it was likely that such genetically restricted suppressor cells were also contributing to post trauma immunosuppression in patients. Tissue typing of patients to allow performance of a histocompatible MLR suppressor assay proved not to be a practical approach. Therefore, we attempted to develop a practical system which would allow detection of patients' genetically restricted T_s in a relatively short time frame. Although MØ were generally not described as targets of T_s, a few reports in the literature appear to suggest that T cells could down regulate MØ function (58). We initially produced an excess of T_s activity by activating a normal peripheral blood cell population with Concanavalin A (Con A). We had already demonstrated that these Con A T cells would suppress in a three way MLR. In our original publication (30), we examined the ability of Con A activated T_s to decrease PA production of syngeneic MØ. We found that T_s could depress MØ PA activity by 40-70 percent when the ratio of MØ to T_s was 2:1. Reduction of the number of T_s and increasing the MØ to T_s ratio to 10:1 reduced the suppression mediated (Tables 8-9). These data indicate that T_s can be assessed by their ability to depress MØ PA function. Interestingly, we found that the Con A generated T lymphocyte population contained suppressors of both the T8+ and the T4+ T cell subset. We concluded from this that this assay could be used to detect both patient T suppressor inducers ($T4^+$) and T effectors (T8). To detect genetically restricted T_s, we utilized the fact that patient MØ PA does not appear to be suppressed in the first 24-48 hours post injury. We had previously determined that these MØ cultured for 2 days in Iscove's media with 15% FBS were still actively producing PA. Initially, we had to explore a number of culture

conditions to find ones which allowed longer term cultures of MØ. These data also established that trauma had not immediately depressed MØ PA synthetic capacity since the MØ in culture until 5 days post injury had normal PA production while the MØ collected from the patient at 5 days post injury had depressed MØ PA production. We could then use the patients' early MØ (held in culture) to assess that same patients' T_s activity. We, therefore, added patient T_s to either normal individuals' MØ (cultured for 3 days) or to the patients own MØ (cultured for 3 days). As can be seen in Table 10, the patients' T_s depressed the PA response of normals and patient MØ populations. The detection of depression of allogenic normal's MØ PA production by patient T_s lead us to question if the mechanism of suppression we were examining was genetically restricted. We consequently examined suppressive activity of the T_s subset defined as being the suppressor inducer (i.e., $CD4^+$ $2H4^+$) population by use of monoclonal antibodies directed to specific T cell phenotypic markers. As discussed in the background, the $T4^+$ T lymphocyte suppressor inducer can be segregated from the $T4^+$ helper inducer by its expression of the surface proteins that react with the monoclonal antibodies 2H4 or leu 8. Initially, we separated normal human PBL T lymphocytes into T helper/inducer and T suppressor/cytotoxic suppressor inducer and helper inducer subsets by panning using leu 8. The results of these experiments are shown in Table 11. We found that most of the suppressive activity for the MØ PA was contained in the suppressor inducer population. The T8 (suppressor/cytotoxic) population was suppressive when derived from concanavalin A (mitogen) activated populations but not when derived from unstimulated T lymphocyte populations. These data imply that selective monoclonal antibody therapy might be useful in moderating the post trauma immunosuppression mediated by excessive T_s.

As we define the different trauma induced defects, we are interested both in how they might be modulated with therapy and how these defects might be rapidly detected in an Army applicable setting. For example, the detected increase in

MØ PGE_2 production post-injury might be modulated by administration of a prostaglandin synthetase inhibitor such as indomethacin or ibuprofen. If the action of the post trauma generated T_s is to suppress T helper cell function, then T helper cell activators like thymosin peptapeptide (TP5) might be able to offset the trauma induced immunosuppression. Conversely, if the immunosuppression is a result of T_s depression of MØ IL-1 production or MØ antigen presenting function, then a monocyte activator which does not increase PGE_2 production might counteract the T_s depressing effect. In a series of experiments, we examined several of these prophylactic modalities. In several of these studies we utilized a guinea pig burn model. In this guinea pig model, a 25% scald burn is administered to the animals. Using this model (Publication #37), we were able to show that both Thymopoetin pentapeptide and indomethacin administered 8-10 hours after thermal injury could partially prevent the loss of immunocompetence in the animals. Neither treatment alone was completely effective and the combination of the two therapies produced the most protection from burn induced immunoincompetence. These data suggest that post burn immunosuppression results from a combination of several host defense system alterations and that combinational immunotherapy may be required to effectively prevent loss of immunocompetence after severe injury. We also examined the immunomodulating effect of low molecular weight dextran (a MØ activator) given as a prophylactic treatment to patients early in their post-injury period. Since dextran is an FDA approved drug used in many clinical settings, we could directly evaluate its immunomodulatory affect in a trauma patient population. Our results (Publication #38) indicated that dextran administration could partially prevent loss of some MØ function post injury. This therapy was only effective in patients with moderate injury severity scores (>25 <39) and seemed ineffective in patients with the most severe injuries. These data again seem to implicate multiple immune system defects in patients with the most severe injuries.

We also began to investigate the effect of bacterial products with MØ "adjuvant" properties as possible immunomodulators. We were aware that LPS had been previously shown to depress some immune functions rather than to stimulate them (59). Consequently, we decided to examine the immunomodulating effect of the bacterial products in our in vitro assays before examining their effect on an in vivo animal model. We first examined peptidoglycan polymers because the synthetic monomer muramyl dipeptide (MDP) is derived from these agents. MDP had been suggested as a possible immunopotentating agent for thermal trauma. When we examined the effect of in vitro peptidoglycan stimulation on MØ functions, we found that although some functions were stimulated (such as leucocyte pyrogen production), other critical functions such as antigen presenting capacity were massively depressed (Publication #39). Consequently, we felt that bacterial like adjuvants were probably not appropriate therapy for immunosuppressed burn victims.

In summary, over the life of this contract, we have made the initial demonstration of the post trauma generation of T suppressor lymphocytes, we have determined that nutritional depletion is not the trigger but rather the result of post injury cellular abberations, we have adapted and developed a number of new assays for assessing trauma patients cellular immune function which include: a measurement of MØ Plasminogen activator production, an assay for MØ procoagulant activity, an assay for patient T suppressor cells which uses a MØ target and requires a 2 day incubation rather than a 6 day incubation, and an assay for patient leukocyte pyrogen production. Using these assays, we have defined a number of crucial defects in both monocyte and T cell functions which are correlated with post trauma immunosuppression. Our current hypothesis is that alterations in crucial MØ T cell interactions may be the underlying mechanism of post trauma immunosuppression. In addition, we have examined several types of prophylactic therapies which are directed toward modulating the monocyte and T cell defects we described. Our data seems to indicate that a

combination of defects may be resulting in the immunocompromised trauma patient syndrome. Effective immunotherapy may require a combination of immunotherapeutic agents to contravene these multiple defects. The assays which we have developed are therefore important in the monitoring of the patients for the efficacy of immunotherapy as well as for detecting post trauma alterations in patient leucocyte function.

References:

1. Cohen SD, Isreal E, Spiess-Muir B, et al: 1981. Plasminogen activator is an apparent lymphocyte mitogen. J of Immunol 126:1415.

2. Chapman HA, Vavrin Z, Hibbs JB: 1983. Coordinate expression of macrophage procoagulant and fibrolytic activity In Vitro and In Vivo. J of Immunol 130(1):261.

3. Miller CL, Graziano CJ, Lim RC: 1982. Monocyte plasminogen activator production. Correlation to immune function. J of Immunol 128(5):2194.

4. Miller CL: 1982. Infection in massively injured patients. In Massive Transfusion in Surgery and Trauma (Collins JA, Murawski K, editors). Alan R. Liss, Inc., New York.

5. Wood JJ, Rodrick ML, O'Mahony JB, et al: 1984. A fundamental immunological deficiency in patients with major burns. Ann Surg 200:311.

6. Usui M, Aoki I, Sunshine GH, et al: 1984. A role for macrophages in suppressor cell induction. J of Immunol 132:1728.

7. Beer DJ, Dinarello CA, Rosenwasser LJ, Rocklin RE: 1982. Human monocyte-derived soluble products(s) has an accessory function in the generation of histamine and concanavalin A-induced suppressor T-cells. J Clin Invest 70:393.

8. Chouaih S, Chatenoud L, Klatzmann D, Fradelizi D: 1984. The mechanism of inhibition of human IL-2 production. II PGE_2 induction of suppressor T lymphocytes. J of Immunol 132:1851.

9. Ninnemann JL, Stockland AE, Condie JT: 1983. Induction of prostaglandin synthesis-dependent suppressor cells with endotoxin: Occurrence in patients with thermal injuries. J of Clin Immunol 3(2):142.

10. Miller CL, Thurston KC, Battey F: 1982. Increased monocyte production of PGE_2 and burn patient hypoimmunity. Proc of the Amer Burn Assoc, May, Boston, Massachusetts.

11. Dinarello C, Marnoy SO, Rosenwasser L: 1983. Role of arachidonate metabolism in the immunoregulatory function of human leukocytic pyrogen/lymphocyte-activating factor interleukin 1. J of Immunol 130:890.

12. Murphy PA, Hanson DF, Simon PL, et al: 1980. Properties of two distinct endogenous pyrogens secreted by rabbit macrophage. In Microbiology (Schlesinger D, editor).

13. Rutherford B, Schenkein HA: 1983. C3 cleavage products stimulate release of prostaglandins by human mononuclear phagocytes In Vitro. J of Immunol 130(2):874.

14. Weiler JM, Ballas ZK, Feldbush TL, et al: 1982. Inhibition of human lymphocyte blastogenesis by C3: The role of serum in the tissue culture medium. Immunol 45:247.

15. Ehrlich MI, Kurshell JS, Blumenstock FA, et al: 1981. Depression of phagocytosis by plasmin degradation products of plasma fibronectin. J Lab Clin Med 98(2):263.

16. Miller CL, Graziano CJ, Lim RC, Chin M: 1981. Production of tissue procoagulant factor by patient monocytes. Correlation to thromboembolic complication. Thromb and Hemo 46(2):489.

17. Chiang NY, Miller CL, Tran MH: 1984. Post-burn depression of complement (C2) levels due to monocyte (MØ) dysfunction. Proc of the Amer Burn Assoc Meeting, San Francisco, California, April 11-14, 1984.

18. Miller CL, Tran MH: 1983. Characterization of C2 generation by human monocytes. 5th International Congress on Immunology, Kyoto, Japan, August 21-27, 1983.

19. Yen SE, Walker WS: 1983. Selective induction of enhanced complement receptor 3 activity on murine macrophage. J Cell Physio 115:61.

20. Johnson WJ, Marino PA, Schreiber RD, Adams DO: 1983. Sequential activation of murine mononuclear phagocytes for tumor cytolysis: Differential expression of markers by macrophages in the several stages of development. J Immunol 131(2):1038.

21. Foris G, Hauck M, Dezso B, et al: 1983. Effect of low-molecular-weight lymphokine components on the Fc and C3b receptor-mediated macrophage functions. Cell Immunol 78:276.

22. Sanchez-Madrid F, Nagy JA, Robbins E, et al: 1983. A human leukocyte differentiation antigen family with distinct α-subunits and a common β-subunit: The lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/MAC-1), and the p150,95 molecule. J Exp Med 158:1785.

23. Onozaki K, Akagawa KS, Haga S, et al: 1983. Role of lymphokines in regulation of macrophage differentiation. Cell Immunol 76:129.

24. Asano Y, Hodes RJ: 1983. T-cell regulation of B-cell activation. I-A-restricted T suppressor cells inhibit the major histocompatibility complex-restricted interactions of T-helper cells with B-cells and accessory cells. J Exp Med 157:1857.

25. Broxmeyer HE, Juliano L, Lu L, et al: 1984. HLA-DR human histocompatibility leukocyte antigens-restricted lymphocyte-monocyte interactions in the release from monocytes of acidic isoferritins that suppress hematopoietic progenitor cells. J Clin Invest 73:939.

26. Tabor DR, Saluk PH: 1983. Differential activation of resident macrophage subsets with two sources of lymphokine preparations. Infec & Immunol 40(1):177.

27. Pelus LM, Saletan S, Silver RT, Moore MAS: 1982. Expression of Ia-Antigens on normal and chronic myeloid leukemic human granulocyte-macrophage colony-forming cells (CFU-GM) is associated with the regulation of cell proliferation by prostaglandin E. Blood 59(2):284.

28. Mustafa AS, Godal T: 1983. In vitro induction of human suppressor T cells by mycobacterial antigens. BCC activated OKT4^{+} cells mediate suppression of antigen induced T cell proliferation. Clin Exp Immunol 52:29.

29. Sathish M, Bhutani LK, Sharma AK, et al: 1983. Monocyte-derived soluble suppressor factor(s) in patients with lepromatous leprosy. Infect & Immunol 42(3):890.

30. Keane RM, Munster AM, Birmingham W, et al: 1982. Suppressor cell activity after major injury: Indirect and direct functional assays. J of Trauma 22(9):770.

31. Miller CL, Baker C: 1979. Changes in lymphocyte activity after thermal injury: Role of suppressor cells. J Clin Invest 63:202.

32. Maghsudi M, Miller CL: 1984. The immunomodulating effect of TP5 and indomethacin in burn induced hypoimmunity. J Surg Res 37:133.

33. Stinnett JD, Loose LD, Miskell P, et al: 1983. Synthetic immunomodulators for prevention of fatal infections in a burned guinea pig model. Ann Surg 198(1):53.

34. Munster, AM: 1976. Post-traumatic immunosuppression is due to activation of suppressor T cells. Lancet 1:1329.

35. Germain RN, Pierres M, Benacerraf B: 1980 The role of macrophages in determining the balance of regulatory T cells specific for L-Glutamic Acid^{60}-L-Alaine^{30} L-Tyrosine^{10} (GAT). In Macrophage Regulation of Immunity (Unanue ER & Rosenthal AS, editors). Academic Press, New York.

36. Goodwin J.S., and Webb D.R: 1980. Regulation of the immune response by prostaglandins. Clin. Immunol. Immunopathol. 15:106.

37. Clowes GHA, Hirsch E, George BC, et al: The significance of altered protein metabolism regulated by proteolysis inducing factor, the circulating cleavage product of interleukin 1. Ann Surg 1985; 202:(4)446-458.

38. Cerami A, and Beutler B.: Cachectic: The dark side of tumor necrosis factor. Cold Spring Harbor Symposia on Quantitative Biol. 1986. LI:625.

39. Hartung HP, Bitter-Suermann D, Hadding U: 1983. Induction of thromboxane release from macrophages by anaphylatoxic peptide C3a of complement and synthetic hexapeptide C3a 72-77. J of Immunol 130(3):1345.

40. Morgan EL, Weigle WO, Hugli TE: 1982. Anaphylatoxin-mediated regulation of the immune respone I. C3a-mediated suppression of human and murine humoral responses. J Exp Med 155:1412.

41. Schopf RE, Hammann KP, Scheiner O, et al: 1982. Activation of human monocytes by both human B1H and C3b. Immunol 46:307.

42. Koopman WJ, Sandberg AL, Washl SM, et al: 1976. Interaction of soluble C3 fragments with guinea pig lymphocytes. Comparison of effects of C3a, C3b, C3c, and C3d on lymphokine production and lymphocyte proliferation. J of Immunol 117:331.

43. Schenkein HA, Genco RJ: 1979. Inhibition of lymphocyte blastogenesis by C3c and C3d. J of Immunol 122(3):1126.

44. Cooperband SR, Badger AM: 1979. Suppressor factors in serum and plasma. In Naturally Occurring Biological Immunosuppressive Factors and Their Relationship to Disease. (Neubauer RA, editor) CRC Press Inc., Baca Raton, Florida.

45. Edington TS, Curtiss LK, Plow EF: 1977. The immunosuppressive activity of plasmic degradation products of human fibrinogen. Proc of Invited Symposium VIII, VI Int Cong Thromb Haem, Philadelphia, p. 170.

46. Harvey HA, Albright C, Lipton A: 1977. The effect of fibrinogen degradation products on In Vitro lymphocyte function. Proc Inv Symposium VIII, VI Int Cong Thromb Haem, Philadelphia, p. 226.

47. Lappin DF, Whaley K: 1982. Prostaglandins and prostaglandin synthetase inhibitors regulate the synthesis of complement components by human monocytes. Clin Exp Immunol 49:623.

48. Alpert SE, Auerbach HS, Cole FS, Colten HR: 1983. Macrophage maturation: Differences in complement secretion by marrow, monocytes, and tissue macrophages detected with an improved hemolytic plaque assay. J Immunol 130(1):102.

49. Lappin D, Whaley K: 1982. Adrenergic receptors on monocytes modulate complement componenet synthesis. Clin Exp Immunol 47:606.

50. Ooi YM, Ooi BS: 1982. Biosynthesis of membrane factor B by mouse peritoneal macrophages. Nature 298:389.

51. Zimmer B, Hartung HP, Scharfenberger G. et al: 1982. Quantitative studies of the secretion of complement component C3 by resident, elicited and activated macrophages. Comparison with C3, C4 and lysosomal enzyme release. Eur J Immunol 12:436.

52. Brade V, Kreuzpaintner G: 1982. Functionally active complement components secreted by guinea pigs peritoneal macrophages. Immunobiol 161:315.

53. Ninnemann JL: PGE_2 regulates lymphocyte stimulation by IL-2. Proc Burn Assoc. 1987; 19:10.

54. Antonacci AC, Calvano SE, Reaves LE: 1984. Autologous and allogeneic mixed-lymphocyte responses following thermal injury in man: The immunomodulatory effects of interleukin 1, interleukin 2, and a prostaglandin inhibitor, WY-18251. Clin Immun & Immunopath 30:304.

55. Luger A, Graf H, Schwarz H, et al: Decreased serum interleukin 1 activity and monocyte interleukin 1 production in patients with fatal sepsis. Crit Care Med 1986;14:(5)458-461.

56. Dinarello LA et al, Human Leukocytic Pyrogen/Interleukin-1: Effects on Natural Killer Cell Activity at Febrile Temperatures. In Prog Immunol V, Yamamuray and Tada T, (eds) 1983: Academic Press Japan, Inc. p.1387-1393.

57. Kupper TS, Green DR: Immunoregulation after thermal injury: sequential appearance of $I\text{-}J^{+}$, LY-1 T suppressor inducer cells and LY-2 suppressor effector cells following thermal trauma in mice. J Immunol 1984. 133(6)3047-3053.

58. Koster FT, Williams JC, Goodwin JS: Cellular immunity in Q fever: Modulation of responsiveness by a suppressor T cell-monocyte circuit. J Immunol 1985. 135:(2)1067-1072.

59. Drapier JC, Lemaire G, Petit JF: 1982. Regulation of plasminogen activator secretion in mouse peritoneal macrophages II. Inhibition by immunomodulators by bacterial origin. Int J Immunopharmac 4(1):21.

LIST OF PUBLICATIONS:

1. Miller CL, Trunkey D: 1977. A defect in immune response induction after thermal injury. J Surgical Research 22:621.

2. Baker C, Miller CL, Tung G, Larsc L, Trunkey D: 1977. Post-operative changes in leukocytes regulating man blastogenic responses. Proceedings of Northern California Surgical Association.

3. Baker C, Miller CL: 1978. Suppressor cells in burn patients' leukocytes. Proceedings of Amercian Burn Association, Burmington, Alabama, 55-56.

4. Baker C, Miller CL, Trunkey D: 1979. Identity of leukocytes which comprise trauma patients' resistance. J of Surg Research 26:478.

5. Miller CL, Baker C: 1979. Changes in lymphocyte activity after thermal injury: Role of suppressor cells. J Clin Invest 63:202.

6. Miller CL, Claudy B: 1979. Suppressor T cells induced by thermal injury. Cellular Immunology 44:201.

7. Miller CL, Baker C: 1979. Development of an inhibitory macrophage after splenectomy. Transplantation Proceedings XI:1460.

8. Miller CL: 1979. Immunological assays as measurements of nutritional status. A review. J Parenteral and Enteral Nutrition 2:554.

9. Miller CL: 1979. Burns and the immune network. J Trauma 19:880.

10. Miller CL, Graziano CJ, Lim R: 1979. Alterations in monocyte functions following trauma. Surgical Form XXX:43.

11. Graziano CJ, Miller CL, Lim R: 1980. The role of monocyte function in alteration of the thrombo-fibrinolytic system after shock. Surgical Forum XXXI:24.

12. Miller CL, Lim R, Graziano CJ: 1980. A monocyte defect after severe thermal trauma. Proceedings of the American Burn Association. San Antonio, Texas.

13. Miller CL: 1980. Alteration in macrophage function following injury. In (Ninnemann JL, ed) Immune Consequences of Thermal Injury. Williams and Wilkins, Maryland.

14. Baker C, Miller CL, Trunkey D: 1979. Predicting sepsis in burn patients. J Trauma 19:20.

15. Baker C, Miller CL, Trunkey D: 1979. Correlation of traumatic shock with immunocompetence and sepsis. Surgical Forum XXX:20.

16. Miller CL, Sheldon GF, Claudy B, Carpenter G: 1977. Evaluation of total parenteral nutrition by in vitro measurement of immune capacity. Surgical Forum XXVIII:81.

17. Miller CL, Peterson S, Sheldon GF, Carpenter G, Fuchs R: 1978. Effect of protein-sparing therapy on immunocompetence in normal protein depleted rats. Surgical Forum XXIX:63.

18. Miller CL: 1980. Effect of nutrition on nonspecific inflammatory and specific immune host defense systems. J Medicine 11(4):267.

19. Miller CL, Linn D: 1980. T suppressor regulation of human monocyte function. Proceedings of IV International Congress of Immunology. Paris, France. July 21-26:4.6.13.

20. Miller CL: 1981. Secondary Immunodeficiency in burns and after surgical trauma. In (Webster A, ed) Clinics in Immunology and Allergy. WB Saunders Co. Ltd., Philadelphia. Vol 1(3):641.

21. Miller CL: 1981. The Immune Response of Burn Injury. J Trauma 21(8):677.

22. Miller CL, Graziano CJ, Lim RC, Chin M: 1981. Production of tissue procoagulant factor by patient monocytes. Correlation to thromboembolic complication. Thrombosis and Hemostasis 46(2):489.

23. Miller SE, Miller CL, Trunkey D: 1982. Immune consequences of trauma. In (Trunkey D, ed) Surgical Clinics of North America: Trauma. WB Saunders Co., Vol 62(1): 167.

24. Trunkey D, Miller CL: 1982. Multiple organ failure and sepsis. In (Narjarian JS, Delaney JF, ed) Emergency Surgery. Year Book Publishers, Chicago.

25. Trunkey D, Miller CL: 1982. Post-Splenectomy Sepsis. In (Flint, L., ed) Surgical Infections. Medical Examination Publishing Co., Inc., New York.

26. Miller CL, Trunkey D: 1982. Immunobiology of sepsis. In (Flint, L., ed) Surgical Infections. Medical Examination Publishing Co., Inc. New York.

27. Miller CL: 1982. Infection in massively injured patients. In (Collins JA, Murawski K, ed.) Massive Transfusion in Surgery and Trauma. Alan R. Liss, Inc., New York.

28. Miller CL, Thurston KC, Battey F: 1982. Increased monocyte production of PGE_2 and burn patient hypoimmunity. Proceedings of the American Burn Association, May, Boston, Massachusetts.

29. Miller CL, Graziano CJ, Lim RC: 1982. Monocyte plasminogen activator production. Correlation to immune function. J Immunology 128(5):2194.

30. Miller CL, Woodley R, Balestra M: 1983. A human T_s subset that suppresses monocyte mediator production. 5th International Congress of Immunology, Kyoto, Japan, August 21-27, 1983.

31. Miller CL, Tran MH: 1983. Characterization of C2 generation by human monocytes. 5th International Congress on Immunology, Kyoto, Japan. August 21-27, 1983.

32. Miller CL: 1983. Monitoring of lymphocyte function. Proceedings of the International Burn Conference, U.S. Army Medical Research & Development Command, Institute of Surgical Research, Fort Sam Houston, Texas, January 19-21, 1983, pp. 142-147.

33. Maghsudi M, Miller CL: 1983. Immunodulators in restoration of burn induced immune defects. Proceedings of the American Burn Association Meeting, New Orleans, Louisiana, March 16-19, 1983.

34. Kudsk KA, Miller CL, Sheldon GF: 1983. Adrenalectomy in the guinea pig: operative and perioperative management. Laboratory Animal Science 33(2):177.

35. Chiang NY, Miller CL, Tran MH: 1984. Post-burn depression of complement (C2) levels due to monocyte (MØ) dysfunction. Proceedings of American Burn Association Meeting, San Francisco, California, April 11-14, 1984.

36. Balestra ME, Miller CL, Wong D, Damato R, Falces E: 1984. Augmented monocyte (MØ) production of leukocyte pyrogen (LP) in severely burned patients. Proceedings of American Burn Association, San Francisco, California, April 11-14, 1984.

37. Maghsudi M, Miller CL: 1984. The immunomodulating effect of TP5 and indomethacin in burn induced hypoimmunity. J Surg Res, Vol. 37.

38. Miller CL, Lim RC: 1985. Dextran as Modulator of Immune Function in Trauma Patients. J. Surg Res. 39:183-191.

39. Gold M, Miller CL, Mishell RI: 1985. Soluble Non Cross Linked Peptidoglycan Polymers Stimulate Monocyte/Macrophage Inflammatory Function. Infection and Immunology 49:731-741.

40. Miller CL, Stahlberg J, Kodys K: 1986. Suppression of MØ Subsets by T Cell Subsets. Sixth International Congress of Immunology, Toronto, Canada. July 6-11, 1986.

41. Miller CL, Lim RC: 1986. Post-Ischemia Immunosuppression in a Mini-Swine Model. Journal of Lab Animal Science 36(4):375.

TABLE 1

REPRESENTATIVE BURN PT IMMUNE PROFILE

Patient	Max % PA Sup	Max % PHA Sup	Age	% Burn	Outcome
			Group I		
Nl	24	6	46	30	No complications Discharged Day 36
We	10	7	22	34	No complications Discharged Day 20
Ou	20	12	23	20	No complications Discharged Day 35
Ha	27	14	32	38	No complications Discharged Day 25
Mu	30	17	39	60	No complications Discharged Day 39
Ba	10	4	64	12	No complications Discharged Day 42
			Group II		
Cr	49	+280	21	40	Strep infection - recovered
Nu	40	+167	46	35	Staph infection - recovered
Ny	33	+250	38	35 (4°)	Pseudomonas infection recovered
Eg	29	+173	29	38	Staph infection - recovered
Te	40	+128	28	37	Pseudomonas infection recovered
At	49	+320	25	55	Staph infection - recovered

(Cont.)

TABLE 1 (Cont.)

Group III

Patient	Max % PA Sup	AGE	Max % PHA Sup	% Burn	Outcome
No	62	82	-89	25	Fatal staph sepsis Day 30 post burn
Fr	64	38	-62	80	Fatal staph pneumonia Day 42 post burn
Oc	73	29	-69	35	Fatal staph sepsis Day 28 post burn
En	61	33	-67	80	Fatal Serratia sepsis Day 17 post burn
Bo	62	34	-82	85	Fatal staph sepsis Day 62 post burn
Fe	90	42	-66	50	Fatal staph pneumonia Day 42 post burn
Ba	62	76	-94	17	Fatal E. Coli sepsis Day 25 post burn
Ge	67	81	-61	25	Fatal pseudomonas pneumonia - Day 20 post burn
Md	72	60	-74	40	Fatal pseudomonas pneumonia - Day 12 post burn
Sh	62	22	-66	60	Resistent pseudomonas Resistent staph Discharged Day 117
Wi	65	54	-70	28	Recurrent staph aureus - Discharged Day 122
Be	64	58	-78	25	Strep pneumonia Strep sepsis Discharged Day 91
Cr	55	86	-74	15	Recurrent strep infection Discharged Day 126
Ma	58	47	-53	35	Recurrent staph infection Discharged Day 58

TABLE 2

Range of C Synthesis Response by Normal MØ After Stimulus with Fc

Plaques/10^6 MØ

Normal	a	b	c	d	e	f	g	h	i	j	k	l	m	$\bar{x}$
2 Days of Culture with Stimulus														
None	10	7	9	2	2	2	6	0	8	6	10	-	-	6±3
Fc	22	32	16	10	4	10	16	9	18	15	20	-	-	16±7
4 Days of Culture with Stimulus														
None	10	10	10	10	15	3	5	9	12	13	10	12	10	10±3
Fc	18	30	20	27	20	12	28	19	17	20	15	20	30	21±5

TABLE 3

Range of C Synthetic Response by Normal MØ After Stimulation With PHA Induced Lymphokines

Plaques /10^6 MØ

Normal	a	b	c	d	e	f	g	h	i	j	k	l	$\bar{x}$
2 Days of Culture With Stimulus													
None	2	7	2	6	6	0	-	-	8	-	-	-	4±3
PHA	2	15	15	17	16	5	-	-	12	-	-	-	12±6
4 Days of Culture With Stimulus													
None	4	10	10	13	5	9	10	10	-	3	12	12	9±3
PHA	4	17	20	12	24	15	12	19	-	15	16	15	15±5

TABLE 4

Failure of PPD to Stimulate Normal Individual's MØ C Production

Plaques/10^6 MØ

Normal	a	b	c	d	e	f	g	h	i	j	$\bar{x}$
2 Days of Culture With Stimulus											
None	2	10	9	2	2	8	6	-	-	-	6±4
PPD	2	8	1	0	1	7	1	-	-	-	3±3
4 Days of Culture With Stimulus											
None	4	10	10	10	13	9	5	12	12	10	10±3
PPD	4	7	8	10	8	9	7	6	9	12	8±2

Decreased C2 Synthesis by Burn Pt MØ
Collected at Various Days Post-Burn

Plaques/10^6 MØ

After 4 Days Culture

Patient / Cont.	Post-Burn Days 1 - 2 Unstim	Post-Burn Days 1 - 2 Stim	Post-Burn Days 5 - 7 Unstim	Post-Burn Days 5 - 7 Stim
$p1/c$	$7/10$	$32/27$	$0/8$	$5/22$
$p2/c$	$9/8$	$24/18$	$2/5$	$2/18$
$p3/c$	$2/10$	$12/20$	$0/10$	$6/18$
$p4/c$	$3/2$	$10/15$	$7/5$	$9/18$

TABLE 6

Correlation of PGE_2 with Massive Increase of PGE_2 at 1-4 Days Post-Burn

Patient	Max PGE_2 1-4 Days	Max PGE_2	Outcome
Group I			
AR	+200	+500	No complication released
PH	+4,113	+10,000	No complication released
Group II			
EL	+725	+11,566	Staph infection recovered
RI	+2,254	+34,503	Staph infection recovered
ZY	+1,404	+22,871	Pseudomonas infection recovered
Group III			
MO	+8,200	+8,270	Succumbed to staph sepsis
MC	+48,090	+48,090	Succumbed to pseudomonas sepsis

TABLE 7

Elevation of MØ LP Production Concomitant
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

TABLE 8

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

TABLE 9

Suppression of MØ PA Concomitant 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

Table 10

Effect of Patient T Cells on MØ Function

Exp 130	LP▲ temp [a]	PA % [b]
Norm MØ	.15	52.6
Norm MØ + Pt T Cell[c]	.20	22.3
Pt MØ	.65	49.9
Pt MØ + Pt T Cell[d]	.85	13.9

Exp 112	LP▲ temp	PA %
Norm MØ	.3	59.5
Norm MØ Pt + Pt Cell	.35	37.7
Pt MØ	.35	21.5
Pt Mø + Pt T Cell	.75	10.5

a. Leukocyte pyrogen (LP) was assessed as change in temperature (▲ temp) of mice injected with 0.3 ml MØ sup.

b. MØ production of Plasminogen activator (PA) is measured in percent specific fibrinolysis.

c. 2×10^6 normal individuals MØ were cocultured 2 days with 2×10^6 patient (pt) T lymphocytes.

d. 2×10^6 patient isotated at day 1-3 post injury were cocultured for 2 days with the Pt T cells collected 4-6 post injury.

TABLE 11

MØ PA FIBRINOLYTIC UNITS PRODUCED BY 5×10^5 MØ CULTURES

	EXP	EXP	EXP	EXP	EXP	EXP
ALONE	41.9	42.2	35.0	21.9	18.0	21.7
+ T_{SI}	22.2	31.4	13.4	13.3	12.1	14.5
+ T_{HI}	41.0	45.5	38.9	21.7	21.7	26.0
+ T_8	24.9	--	35.6	22.7	12.8	18.1
+ T_{SI} & T_8	24.9	35.9	15.3	--	12.0	--
+ T_{HI} & T_8	41.7	--	25.8	18.9	17.4	--

DISTRIBUTION LIST

4 copies	Commander Letterman Army Institute of Research (LAIR), Bldg. 1110 ATTN: SGRD-ULZ-RC Presidio of San Francisco, CA 94129-6815
4 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-RMI-S Fort Detrick, Frederick, Maryland 21701-5012
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDAC Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Services 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

END

12-87

DTIC