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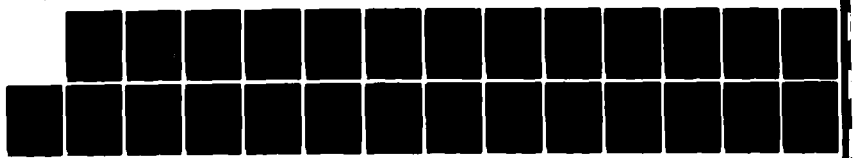

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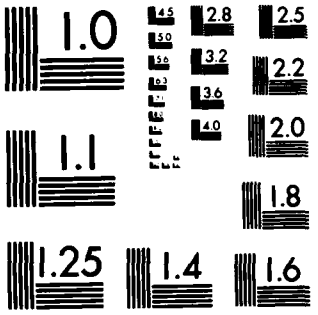

# STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY THERMAL INJURY

ANNUAL PROGRESS REPORT

Carol L. Miller, Ph.D.

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## Introduction

The high incidence of fatal septicemia associated with severe thermal injury is believed to result from loss of immunocompetence. This laboratory has been able to identify those burn patients who are at greatest risk for developing fatal sepsis by detecting the loss of certain immune functions by cells of these patients. Direct burn induced immune dysfunction can result from aberrations in any of the three general types of leukocytes which cooperatively mediate the generation of immune function. These three leukocyte subpopulations are the antigen specific bone marrow-derived (B) cell, the antigen specific thymus-derived (T) cell, and a third extremely heterogenous population of leukocytes- the monocyte or macrophage (MØ).

This report describes the results of this year's experiments to reduce the post burn incidence of fatal sepsis by (1) rapidly identifying and segregating those individuals that are at greatest risk of sepsis; (2) delineating the nature of the burn induced immune defect; and (3) characterizing those mechanisms by which thermal injury causes immune aberrations. Understanding of these mechanisms may allow development of far forward prophylactic measures which could prevent thermal injury from inducing immune defects. Experimental data derived from our patient studies have allowed us to develop assays for detecting early immune anomalies and to delineate the cell type(s) involved in these aberrations. Our murine model has been primarily utilized to characterize the mechanisms by which thermal injury causes the development of immune defects.

The research for this contract year has focused on burn induced alteration in monocyte (MØ) function which occurs within the first 2-4 days post injury. We postulate that it is these early changes in MØ activities that unbalance the immune network away from immunocompetence and toward excessive regulation and hypoimmunity.

The monocyte population appears to be divided into facilitory and inhibitory subsets just as the T lymphocyte population is segregated into helper and suppressor cells ( 1-3 ). A complex reciprocal interaction occurs between facilitory MØ and T helper cells ( 3,4 ). Recent data indicates that a similar reciprocal interchange occurs between inhibitory MØ and suppressor T cells ( 5-8 ). We have spent this contract year devising and defining assays for measuring early MØ functions and determing which if any of the MØ activities we monitor are correlated to MØ facilitory or MØ inhibitory immune functions. The monocyte functions as well as the immune functions of thermally injured patients are assessed every 3 days from admission to release or demise. Alterations in these MØ activities are determined in comparison to the patients' own initial MØ activity

level and to the established "normal" level. The patients' monocytes are monitored for their production of plasminogen activator (PA), tissue procoagulant factor (TF) and lysozyme (Ly). MØ plasminogen activator production has been suggested as paralleling MØ facilitory activity in murine systems ( 9,10 ). We have examined the correlation of human facilitory MØ activity to MØ PA production. High level TF generation is an aberrant monocyte activity not seen in normal human MØ populations. Lysozyme synthesis is a general indicator of MØ viability and it appears to be unaffected by most specific inhibitory cells or molecules ( 11).

We have previously reported that there is an aberrant increase in inhibitory MØ after severe trauma and splenectomy ( 12,13 ). Any rise in inhibitory MØ (inhib MØ) would severely depress immunocompetence since inhib MØ prevent the proliferation of T helpers, B cells, and also interfere with facilitory MØ activity. Consequently, monitoring of burn patients' MØ functions would contribute not only to evaluation of the patients' immune status, but also to determination of how burns mediate decreased immunocompetence.

## Methods

### Human studies

Patients with greater than 30% full thickness burns are the primary donors of abnormal leukocytes. Leukocytes are obtained by venipuncture from consenting patients. Normal volunteers are donors of control human leukocytes. Appropriate safety precautions are always observed. Minors, prisoners, pregnant women and the mentally handicapped are excluded as donors. Mononuclear cells are isolated from the peripheral blood (PB) by Ficoll-Hypaque gradient centrifugation ( 14 ). Patient mononuclear populations can be further depleted of T cells, monocytes and/or B cells. The T lymphocytes are depleted by removing the cells binding to neuraminidase treated sheep red blood cells (SRBC) on a Ficoll-Hypaque gradient ( 15 ). Monocytes are removed by passing the mononuclear population over Sephadex G-10 columns ( 16 ). The B cells can be removed by nylon wool filtration of the cell population ( 17 ).

We monitor the ability of patient and normal mononuclear cell populations to respond to phytohemagglutinin (PHA) ( 18 ). This non-specific mitogen response requires the cooperative interaction of monocytes and T cells ( 19 ).

Monocytes (MØ) are isolated from the Ficoll-Hypaque purified mononuclear cell populations by the Ackerman and Douglas rapid adherence technique ( 20 ). These isolated monocytes are then examined for the production of PA, their level of tissue procoagulant factor (TF) activity and their synthesis of lysozyme. In the PA assay, patients' and normal controls' isolated MØ are placed onto $^{125}$I-fibrin plates and cultured 18 hours either in the presence of acid treated fetal bovine sera (AT-FBS) or soybean trypsin inhibitor (SBI), an inhibitor of plasmin. After all the PA is released in these cultures, the cells are washed and fresh AT-FBS media or SBI

media is added for an additional 24 hour incubation period. The amount of fibrinolysis initiated during this second incubation period is then measured. Monocyte numbers have been adjusted to produce approximately 25-35 fibrinolytic units for normal individuals ($4 \times 10^5$ isolated MØ). Simultaneous to our assessment of burn patients monocyte's PA synthesis, we also assay their production of TF and lysozyme. TF production is measured using the Rickle's assay and lysozyme production is measured using the Schill and Schumacher Lysozyme Plate test (21,22). Human mononuclear cells are separated into T, B or MØ subpopulations and the interaction between these subpopulation is evaluated. T cells are segregated and isolated by formation of rosettes with SRBC ( 15 ). The effect of suppressor T cells or of inhibitory MØ is assessed by mixing purified autologous cell populations and assessing the effect of one cell type (i.e. suppressor T cell) on another cell population's function.

We have detected burn induced aberrations in the immune regulatory functions of patients' cells using a modification of the classical mixed lymphocyte response (MLR). In our MLR system a highly responsive combination of cells from two normal individuals are cultured in a "one way" MLR ( 23 ). In this assay, one group of the normal's cells are pretreated with mitomycin C (MC) to prevent their division ( 18 ). Consequently, this "one way" MLR assay measures the ability of one group's normals cells(Responder=R) to proliferate in response to the foreign histocompatibility antigens on another normal's cells (Stimulator=S). We compare the effect of adding either burn patient cells or MC treated responder cells, on the amount of proliferation in the MLR cultures.

Data Calculation and Statistical Analysis

The data presented for patient and normal's PA production is always from the second incubation interval. All supernate CPM's of $^{125}$I-fibrin are corrected for media and non-specific radioactivity release by subtraction of CPM's from no cell controls. The CPM's of $^{125}$I-fibrin in the supernates from the lines containing cells in 100 ug SBI are subtracted from the CPM's of lines containing the cells in AT-FBS. This corrects for any $^{125}$I-fibrin lysed by other than plasmin mediated mechanisms. This corrected AT-FBS CPM is then divided by the total $^{125}$I-fibrin CPM's present to derive the percent specific plasmin mediated lysis. This value is computed for patient cells collected every four days post injury. The mean and standard deviation of PA production by MØ from 43 normal individuals tested repeatedly was $25 \pm 8.4$, and their own initial (day 1) values. A Student's T test was used to determine significant differences. The TF activity of sonicates from $10^5$ MØ was calculated in thromboplastin equivalent units by comparison of the shortened thromboplastin time to a control brain thromboplastin standard curve.

Human peripheral blood mononuclear cell populations differ from individual to individual in their percentage of MØ, T and B cells and their degree of immune reactivity. Human immune functions have been suggested as being controlled by immune response genes analogous to those described in animal systems ( 24, 25 ).

Consequently, the "normal" levels of MØ PA production, mitogen responsiveness, MØ TF generation, lysozyme production, and MØ $PGE_2$ activity vary for each patient and within the normal control groups. The baseline levels of each individual's MØ and T cell activites are not randomly distributed. Some individuals are low and some are high responders. This nonbinomial distribution of the MØ and T cell parameters necessitates the use of non-parametric statistics when analyzing patients' data. We utilize the Wilcoxon test for evaluating the statistical significance of alterations in patients' mitogen, PA, and TF assays. We utilize Spearman's correlation coefficient for determing the degree of interdependence between the various MØ and T cell parameters.

Murine experiments

In each experiment, Balb/k or $C_{57}Bl/6$ inbred mice of 16-18 weeks of age are obtained from the Diablo Mouse Colony at the University of California at Berkeley. Under light Metofane anesthesia, littermate mice are shaven and then divided into two groups. One group receives a 10-20% scald burn with 92°C water for 5 seconds (experimental mice) while the second group is not burned (sham-control mice). At specific times after injury, 2-4 mice from each group are sacrificed. These animals' spleens are removed, teased into single cell suspensions and cultured *in vitro* with sheep erythrocytes using a modification of the Mishell-Dutton culture technique ( 26 ). We monitor thermal injury effects on immunocompetence by measuring the formation of specific antibody forming cells (AFC). This system facilitates detection of cell immunoregulatory interactions.

Monocytes and or T leukocytes are depleted or isolated from the burned mice. Purified, syngeneic, normal or control leukocytes are added to these depleted thermally injured populations. In this manner, normal monocytes or T cells are supplied to the immunodepressed burned mouse's cells. These experiments examine whether supplying normal, functional MØ or T cells restore the ability of the thermally injured leukocyte population to generate normal numbers of specific AFC. Monocytes are depleted from leukocyte populations according to the method of Ly and Mishell ( 16 ). T cells are lytically removed from leukocyte populations by treatment of the splenocytes with anti-T cell antisera and complement ( 27 ). Leukocyte populations are depleted from splenocyte populations by passing the cell preparations over nylon wool columns (17). These nylon wool columns remove MØ as well as B cells.

The *in vitro* generation of AFC is assayed using the slide modification of the Hemolytic Plaque Assay (28). Leukocyte recovery from cultures is determined by counting a sample of the harvested, cultured cells on a Coulter Counter (Model ZH). The number of AFC are calculated for each pool of duplicate background plaques and expressed as AFC/$10^6$ recovered spleen cells. Allogeneic conditioned media is produced as described (29). In order to augment MØ function, 2-mercaptoethanol (2ME) is sometimes added to cultures at a final concentration of $5 \times 10^{-5}$ M.

## Results and Discussion

This contract year, we have monitored post burn alterations in 8 patients in the 20 - 55 year age group who sustained greater than 30% 3rd degree burns. Of this group, two patients succumbed to overwhelming sepsis. One patient survived 8 days post injury and the other 16 days. Only cells from those two burn patients who ultimately developed septicemia exhibited any reduced MØ PA and depressed mitogen responsiveness. The data from one of these burn patients is illustrated in Fig. 1. As can be seen, there appears to be a direct correlation betweeen PHA mitogen hyporesponsiveness (a measure of MØ - T immune cooperation) and depressed MØ PA responses. However, two patients represent too small a group to convincingly establish that MØ PA function reflects MØ immune facilitory activity. Consequently, we examined the correlation between MØ PA and MØ facilitory functions in another severe trauma patient group where we have larger patient numbers.

We have examined the immunocapacity of 20 patients who sustained splenectomy for trauma. Fourteen of these patients were studied every 3 days for greater than 20 days. As a first step in employing the PA assay as a system for monitoring patient MØ function, we extensively examined the MØ PA responses of 22 normal individuals. Each individual was repeatedly assayed (4 - 12 tested) over a 6 month period to evaluate the variability in normal human MØ responses. Table 1 summarizes these results and includes the simultaneously assayed PHA responses of these individuals. Although the MØ PA response of murine cells seems to parallel facilitory MØ activity, this relationship has not been demonstrated in human cell populations. We chose to examine the relationship between MØ PA and MØ facilitory function in the splenectomized trauma patient. Because of the number of splenectomized patients available we were able to examine a greater number of individuals who might exhibit MØ defects over a shorter time period. We are, however, continuing to study the correlation between MØ PA and MØ facilitory immune function in each severely burned patient admitted.

Out of the fourteen splenectomy trauma patients examined, 7 individuals experienced a significant ($p < 0.005$ by Wilcoxon) depression in their MØ PA function. These data are illustrated in Figure 2a and Figure 2b. Seven individuals experienced no significant alteration in their PA response. Six of these patients' MØ PA data are illustrated in Figure 2c. The peak of MØ depression in these splenectomy trauma patients appears at 8 - 13 days post injury. It might be argued that the loss of the monocyte PA function is an artifact of the surgical manipulation which may cause a migration of PA producing MØ to other sites after splenectomy. If this were the case, one would expect to see all patients with major surgical manipulation show this same depression. The data in Figure 3, illustrate the PA response of three major surgical patients over time. One of these patients is a liver resection for removal of a hepatoma; one is a patient splenectomized for blunt trauma injury; and the third is a patient who underwent severe multiple trauma and splenectomy. Only the

splenectomized multiple trauma patient exhibited a decreased PA response. As can be seen in Figures 4 and 5, the appearance of a MØ defect may slightly precede the development of PHA hyporesponsiveness. Interestingly, MØ production of TF (a normally minimal function) increases in trauma patients who are experiencing reduced immunocompetence. All of the 7 severely injured individuals who experienced depressed MØ PA responses exhibited PHA hyporesponsiveness and developed septic and/or hypercoagulability complications. None of the seven splenectomy patients whose MØ PA and PHA responses did not deviate significantly from their base line ($p > 0.1$ by Wilcoxon) developed nosocomial episodes or coagulopathology. Table 2 illustrates the correlation of PHA hyporesponsiveness to depression of the MØ PA response for all 14 of the splenectomized trauma patients. As can be seen, these two host defense parameters are highly correlated ($r_s = 0.823$). As portrayed by Figure 6, there also appears to be an excellent correlation over time between decreased MØ PA activity and PHA hyporesponsiveness in these severely injured patients.

One explanation for the loss of MØ PA and development of PHA hyporesponsiveness in these patients could be that overall MØ viability or functionality is decreasing. It has been suggested that post injury increased steroid concentrations or elevated levels of other toxic metabolic substances are cytolytic for mononuclear cells. In contrast, our own hypothesis is that the mononuclear cells are not destroyed but that critical regulatory balances between cell subsets are altered. If there were a general depletion in MØ viability or numbers, then all MØ functions should be reduced. We have monitored not only MØ PA but also MØ production of TF and lysozyme in our patient studies. MØ TF generation tends to be low (ave $4.3 \pm 2.3$ units) in normal humans. However, MØ TF generation can be increased in response to endotoxin or C split products (30,31). Lysozyme is a general macrophage product whose production appears to be fairly consistent. As illustrated in Table 3, we examined TF, PA and lysozyme production from the splenectomized trauma patients. Those patients whose MØ exhibited decreased PA functions had significantly ($p < 0.001$) increased TF activity ($\bar{X}$ increase = +32 units) and unchanged lysozyme production. There was a significant negative correlation between increased TF and decreased PA in these patients' MØ population ($r_s = 0.783$).

From this year's work, we have interpreted our patients data as indicating the following. Decrease of patients' MØ PA function is an indicator of depressed facilitory MØ function as well as reduced fibrinolysis. A patient with such reduced host defense capacity is at increased risk of both septic and hypercoagulable complications. The loss of MØ PA and facilitory function are not the result of reduced MØ numbers or MØ viability. The increase in MØ TF activity reflects a metabolically active and viable MØ population in which the subset ratio has been shifted away from facilitory MØ toward another MØ subpopulation (inhibitory ?). We do not know if the inhibitory MØ population is now increased at the expense of the facilitory MØ subset. $PGE_2$ production is

the marker for the inhibitory MØ subset ( 32). Experiments are now underway to determine if increased MØ TF activity is correlated to augmented MØ $PGE_2$ production. We have only recently instituted the $PGE_2$ radioimmunoassay in this laboratory. This assay ($PGE_2$) will play a large part in our experiments of the coming year.

Another aspect of burn mediated changes in immunocompetence has been investigated this year. We have previously demonstrated that burn patients who are predisposed to septic death developed excessive T suppressor activity (33 ). One of the questions that has been repeatedly raised about this data is what relevance augmented suppressor T cell activity has to reduction in host defense. Besides the obvious disruption of the immune system, we wondered if these suppressor T cells could affect host defense activities that are normally considered "inflammatory functions." T suppressors are known to inimically effect MØ facilitory immune function. It is possible, therefore, that these T suppressors could depress MØ inflammatory activities such as PA production and/or production of factors which control PMN migration and maturation (CSF, LAF). In the following set of experiments we artificially generated (Con A stimulation) suppressor T cells in normal human PB mononuclear populations. We then tested the ability of these $T_s$ to depress MØ PA function, MØ TF function and lysozyme production. As portrayed in Table 4, the addition of Con A $T_s$ to purified, isolated monocytes significantly reduced their ability to produce PA. Addition of cultured normal cells or fresh normal cells had no such depressive effect. The data in Table 5 demonstrate that this depression of MØ function is mediated by the added $T_s$ not by any addition of inhibitory MØ or by a dilution of the MØ population. As can be seen in Table 6, the Con A $T_s$ did not adversely affect MØ TF or lysozyme function. These data indicate that $T_s$ can specifically depress one MØ subset without disrupting the function of other MØ subsets. The data also indicate that $T_s$ have the ability to interfere with the pathways of the inflammatory system as well as the specific immune system. These data support our hypothesis that it is the monocyte connections between the specific and the nonspecific inflammatory systems that are most vulnerable to excessive regulation. The data also strengthen our contention that alterations in the functions of these interconnecting MØ can adversely affect the function of both host defense systems. Therefore, it is critical to investigate the role of inhibitory MØ in these $T_s$ - facilitory MØ interactions and to determine which MØ functions in both the specific immune and the inflammatory system can be depressed by regulatory T cells.

In order to explore some of these MØ - T cell interactions after thermal insult, we need to utilize animal models. Our standard murine model has produced some interesting data relating the loss of MØ PA function to the development of immune aberrations in the AFC response. These data are summarized in Table 7. As can be seen, these data suggest that MØ PA dysfunction precedes the development of immune hyporesponsiveness. We are presently using cell mixing experiments to determine at what post trauma

period each cell dysfunction first appears. We wish to determine if a MØ dysfunction precedes $T_s$ development which then intensifies the MØ dysfunction. We are delineating when (if ever) augmented inhibitory MØ activity appears after thermal insult and what the consequences of this appearance are to development of host defense dysfunction.

Characterization of how burns initially trigger MØ dysfunction is a major focus of this laboratory's experimentation. As detailed in Introduction, considerable data has been recently accumulated demonstrating an interrelationship between elevated steroid concentrations, increased MØ $PGE_2$ production, agumented suppressor T cell activation and depressed facilitory MØ function ( 5-8 ). This laboratory has recently introduced an adrenalectomized guinea pig model to investigate the interrelationship between these activities and their alterations after thermal insult. The guinea pig is an optimal model for steroid studies. Cortisol is the major glucocorticoid produced by both man and guinea pigs. Additionally, both the guinea pig complement cascade system and immune system are well described and have been found to be very similar to those of humans. The adrenalectomized guinea pig model we are utilizing is described in a publication we are submitting to Endocrinology.

Our expanded assay systems for assessing MØ function and examining steroid and $PGE_2$ effects enable us not only to monitor patients' host defense status but also to probe the mechanisms by which burns cause reduced host resistance.

## Conclusion

In summary, our laboratory has produced substantial data indicating that MØ dysfunction does appear in those severely burned patient who will eventually develop severe or fatal septicemia. This MØ dysfunction is characterized by a loss of MØ PA production and a rise in MØ TF generation. Loss of patient MØ PA function appears to correlate to a development of T suppressor cells and mitogen hyporesponsiveness. MØ PA appears to be a property of the facilitory MØ subset and to be a marker for facilitory MØ functionality. The data from our human systems also suggest that once excessive T suppressor cells are activated after thermal injury, they can then further disrupt vital facilitory MØ functions. Our data from the murine system supports the concept that unbalancing of the immune network toward too much regulation (ie, increased suppressor T cells) is preceeded by and probably a result of depressed monocyte facilitory immune function.

TABLE 1

Variation in PHA, PA, TF, and lysozyme response parameters of 0 and/or YC from different normal individuals.

| Normals | PHA Ave ± CPM | PA (% Fibrinolysis) | TF Throm. Eq. Units | Lysozyme Units |
|---|---|---|---|---|
| DA | 84,936 ± 7,369 | 23.3 ± 0.2 | 5.0 ± 2.5 | 11.9 ± 2.8 |
| DB | 67,335 ± 6,768 | 20.1 ± 2.0 | 4.3 ± 0.3 | 9.9 ± 0.7 |
| EF | 31,585 ± 1,613 | 17.6 ± 0.1 | 4.8 ± 1.3 | 11.3 ± 1.0 |
| FK | 61,427 ± 3,426 | 26.8 ± 2.2 | 8.5 ± 2.8 | 12.5 ± 2.7 |
| KL | 53,664 ± 2,288 | 28.3 ± 2.1 | 7.4 ± 3.3 | 9.2 ± 2.4 |
| EL | 35,299 ± 3,395 | 18.7 ± 1.4 | 7.6 ± 3.4 | 7.5 ± 1.1 |
| MH | 35,778 ± 1,205 | 19.3 ± 1.3 | 4.8 ± 0.5 | 9.5 ± 2.9 |
| QC | 52,138 ± 1,014 | 26.4 ± 2.8 | 3.8 ± 2.4 | |
| BF | 63,617 ± 2,024 | 26.6 ± 2.4 | 1.1 ± 0.1 | 9.0 ± 3.4 |
| RW | 49,246 ± 889 | 26.1 ± 2.3 | 8.2 ± 2.3 | 18.7 ± 2.8 |
| GS | 57,963 ± 3,539 | 23.1 ± 2.6 | 2.7 ± 2.2 | 10.0 ± 1.6 |
| MT | 92,694 ± 6,789 | 26.0 ± 2.7 | 3.8 ± 1.8 | 9.4 ± 3.0 |
| DL | 46,047 ± 4,522 | 25.5 ± 2.1 | 6.8 ± 3.7 | 10.0 ± 2.7 |
| JM | 46,253 ± 3,872 | 25.2 ± 1.5 | 3.6 ± 1.5 | |
| TM | 83,001 ± 6,394 | 30.9 ± 3.8 | 5.2 ± 2.6 | 10.3 ± 3.4 |
| YV | 27,444 ± 2,845 | 18.8 ± 2.1 | 2.6 ± 1.9 | 4.9 ± 1.2 |
| AM | 92,235 ± 8,306 | 25.4 ± 3.7 | 2.9 ± 2.5 | 12.0 ± 2.8 |
| RM | 34,144 ± 1,003 | 19.5 ± 1.3 | 6.1 ± 1.5 | |
| FD | 35,360 ± 3,063 | 23.9 ± 3.4 | 7.4 ± 3.7 | 8.4 ± 3.4 |
| CG | 32,355 ± 2,263 | 17.4 ± 2.8 | 6.4 ± 1.3 | 11.5 ± 2.1 |
| CM | 72,413 ± 1,516 | 21.4 ± 3.4 | 6.7 ± 2.8 | 6.8 ± 2.5 |
| MS | 68,429 ± 1,761 | 24.6 ± 3.5 | 2.7 ± 1.5 | |

TABLE 2

Correlation[a] of Deceased Monocyte PA Activity to Depressed PHA Response of the Same Mononuclear Cell Population.[b]

| Splenectomy Patient | Minimum PA response | PHA response on day of lowest[d] PA activity | Minimum PHA response[e] |
|---|---|---|---|
| | (A) | (B) | (C) |
| BF | 1 | 35 | 35 |
| MF | 11 | 36 | 34 |
| RM | 11 | 42 | 37 |
| SM | 13 | 48 | 44 |
| MM | 7 | 18 | 18 |
| EM | 9 | 37 | 35 |
| SC | 12 | 40 | 38 |
| SZ | 31 | 84 | 84 |
| BM | 22 | 87 | 87 |
| KM | 25 | 78 | 78 |
| BO | 20 | 83 | 82 |
| MZ | 22 | 88 | 85 |
| JM | 29 | 79 | 79 |
| KP | 21 | 85 | 85 |

[a]Correlation between Column (A) and Column (B) determined using Spearman's rank correlation coefficient $r_s = 0.823$ ($p < 0.001$, 2 tailed). Column (A) and (C) are also correlated to the same degree.

[b]Monocytes were isolated from the mononuclear cell population assessed in the PHA assay.

[c]Lowest PA response in % fibrinolysis of $4 \times 10^5$ isolated monocytes collected during the 8-15 days post injury interval.

[d]PHA response of mononuclear cell population at time PA response was lowest. PHA data as percent of baseline response.

[e]The post injury day of minimal PHA responsiveness often did not coincide with the day of lowest PA activity, but occurred 2-3 days post trauma later.

TABLE 3

TF[a] and lysozyme parameters of monocytes from splenectomy patients.

| Splenectomy Patient | Maximal depressed PA | TF Activity (Eq. units) | Lysozyme units |
|---|---|---|---|
| **Patients with a significant decrease in PA response** | | | |
| | A[c] | B[d] | C |
| BF | 22 → 1 | 68 | 11.7 |
| MF | 25 → 11 | 30 | 13.2 |
| RM | 29 → 11 | 33 | 19.5 |
| SM | 32 → 13 | 26 | 7.6 |
| MM | 26 → 7 | 45 | 12.3 |
| EM | 22 → 9 | 33 | 11.0 |
| SC | 26 → 12 | 40 | 9.9 |
| **Patients with no decrease in PA response** | | | |
| Splenectomy Patient | Maximal change in PA | TF Activity (Eq. units) | Lysozyme units |
| SI | 34 → 31 | 11.0 | 8.3 |
| BM | 22 → 26 | 5.0 | 10.0 |
| KM | 25 → 33 | 12.0 | 9.4 |
| BO | 20 → 26 | 10.0 | 9.0 |
| MI | 22 → 21 | 2.4 | 9.0 |
| JM | 29 → 32 | 4.8 | 11.2 |
| KF | 21 → 29 | 1.1 | 10.7 |
| $\bar{X}$ Normals = | | 5.2 ± 2.1 | 10.2 ± 3.1 |

[a]TF and lysozyme values as tested on day of maximal depression.

[b]Lysozyme - one unit of nuramidase activitv defined as ΔA450 of 0.001 per minute at pH 7.0 at 25°C. using Micrococcus lysodeikticus as substrate.

[c]PA activity (Column A) is inversely correlated to TF production (Column B) $p < 0.002$ 2 tailed $r_s = 0.783$.

[d]TF versus lysozyme (Column C) not correlated and Lysozyme versus PA not correlated $p > 0.5$.

TABLE 4

## SUPPRESSION OF PA RESPONSE BY CON A CELLS

| | PA response of 4 X $10^5$ MØ incubation with: | | | |
|---|---|---|---|---|
| added cells | exp. 3 | exp. 7 | exp. 8 | exp. 15 |
| fresh P.B.M. | 27.1 | 34.6 | 34.9 | 28.7 |
| cultured P.B.M. | 33.2 | 43.4 | 39.8 | 38.3 |
| Con A P.B.M. | 17.8 | 14.2 | 13.4 | 11.1 |

PA - production of plasminogen activator in fibrinolytic units

P.B.M. - peripheral blood mononuclear cells

## CON A INDUCED SUPPRESSOR T CELLS ARE MEDIATING LOSS OF MØ PA FUNCTION

Response of 4 x $10^5$ MØ after incubation with:

| added cells | exp. 18 | exp. 22 | exp. 23 | exp. 28 |
|---|---|---|---|---|
| Cul MØ | 24.8 | 22.6 | 41.8 | 28.1 |
| Con A MØ | 26.2 | 19.2 | 42.1 | 30.8 |
| Cul T | 25.4 | 31.9 | 43.6 | 38.0 |
| Con A T | 7.9 | 8.4 | 4.2 | 10.4 |

TABLE 5

Cul MØ—non-rosetting adherent cells from 48 hr. cultured mononuclear population

Con A MØ—non-rosetting adherent cells from 48 hr. cul and Con A

Cul T—E rosette pos cells from 48 hr. cul

Con A T—E rosette pos cells from 48 hr. cul and Con A

## CHANGE IN MØ FUNCTION AFTER INCUBATION WITH CON A SUPPRESSOR CELLS

| Δ PA RESPONSE | Δ LYSOZYME RESPONSE | Δ TF RESPONSE |
|---|---|---|
| decreased 65% | increased 4.0 units | increased 12 units |
| decreased 68% | increased 2.1 units | increased 14 units |
| decreased 66% | increased 3.1 units | increased 7 units |
| decreased 67% | increased 2.7 units | increased 17 units |
| decreased 68% | increased 2.6 units | increased 7 units |

TABLE 6

Table 7

Comparison of Altered MØ PA Activity to Depressed AFC Response in Burned Mice

| Day post injury | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| % Sham PA | 55 | 38 | 56 | 69 | 94 |
| % Sham AFC | 90 | 80 | 55 | 45 | 32 |

FIGURE 1

POST INJURY CHANGES IN LYMPHOCYTE AND MONOCYTE ACTIVITIES AFTER SEVERE THERMAL TRAUMA

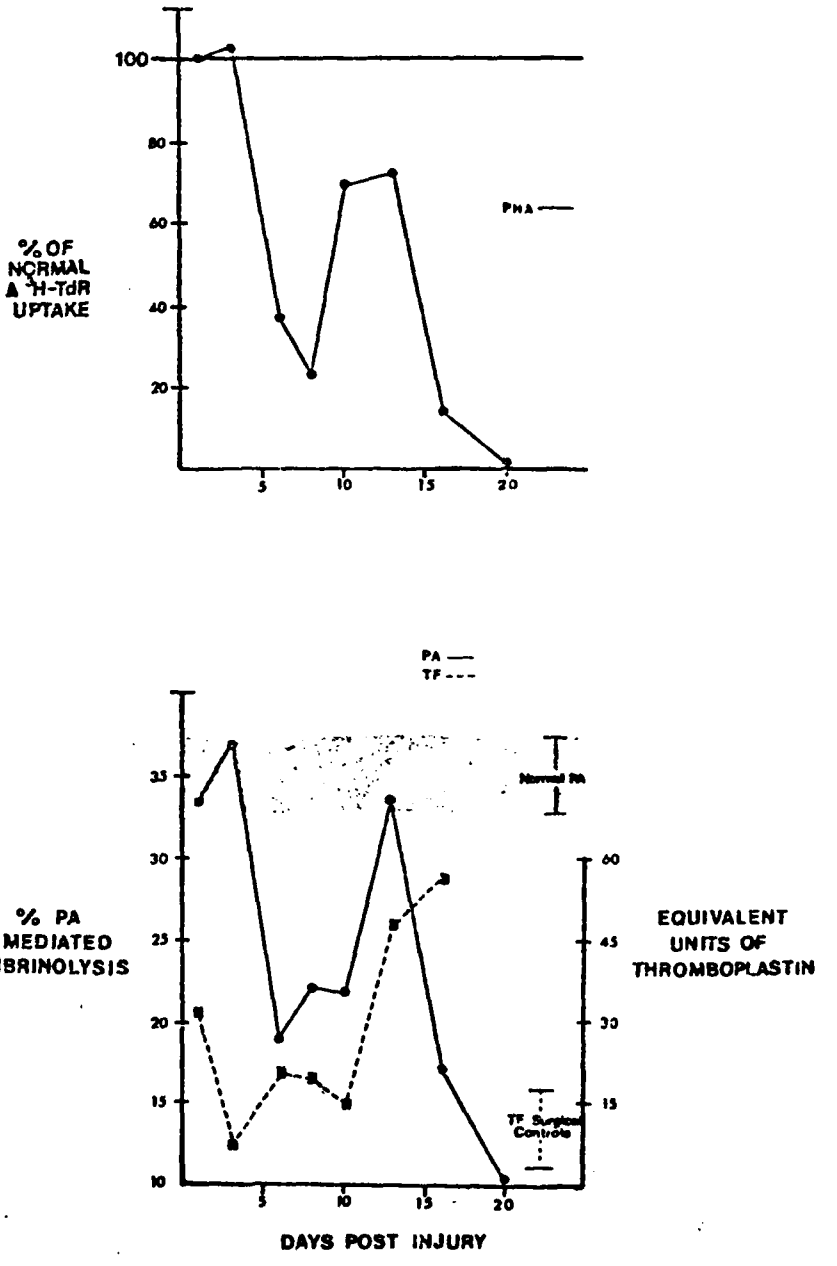


FIGURE 2

ALTERATION IN MØ PA FUNCTION IN 14 PATIENTS AFTER SEVERE TRAUMA

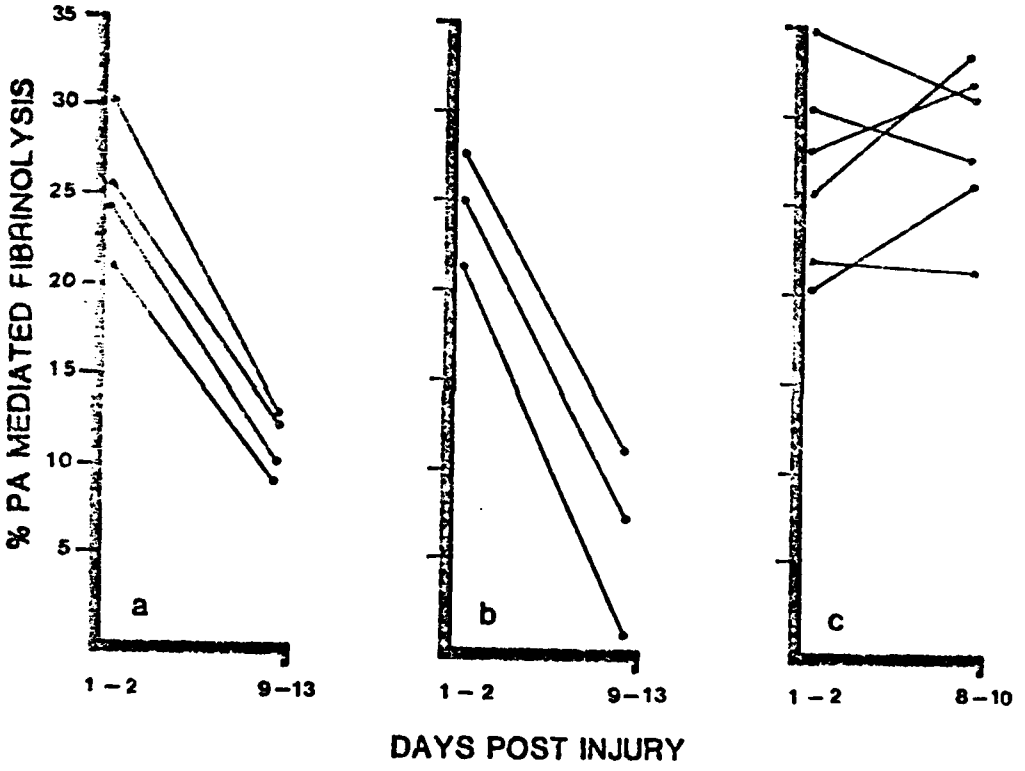


FIGURE 3

COMPARISON OF MØ PA PRODUCTION IN THREE PATIENTS

AFTER MAJOR SURGICAL MANIPULATION

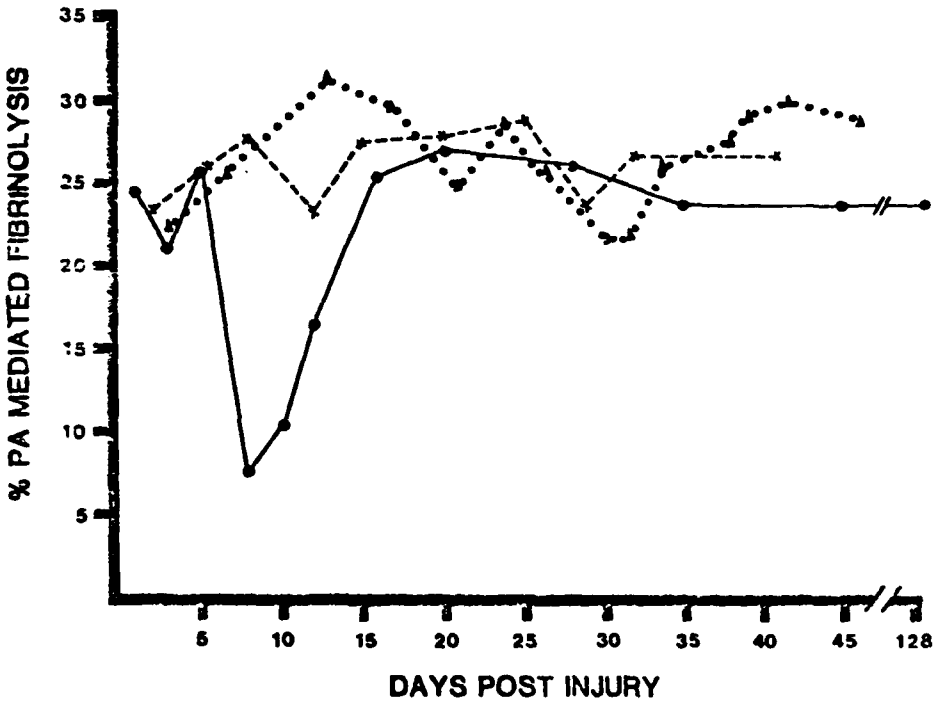


FIGURE 4

COMPARISON OF PHA HYPORESPONSIVENESS TO ALTERED MØ TF ACTIVITY IN A SEVERELY INJURED PATIENT

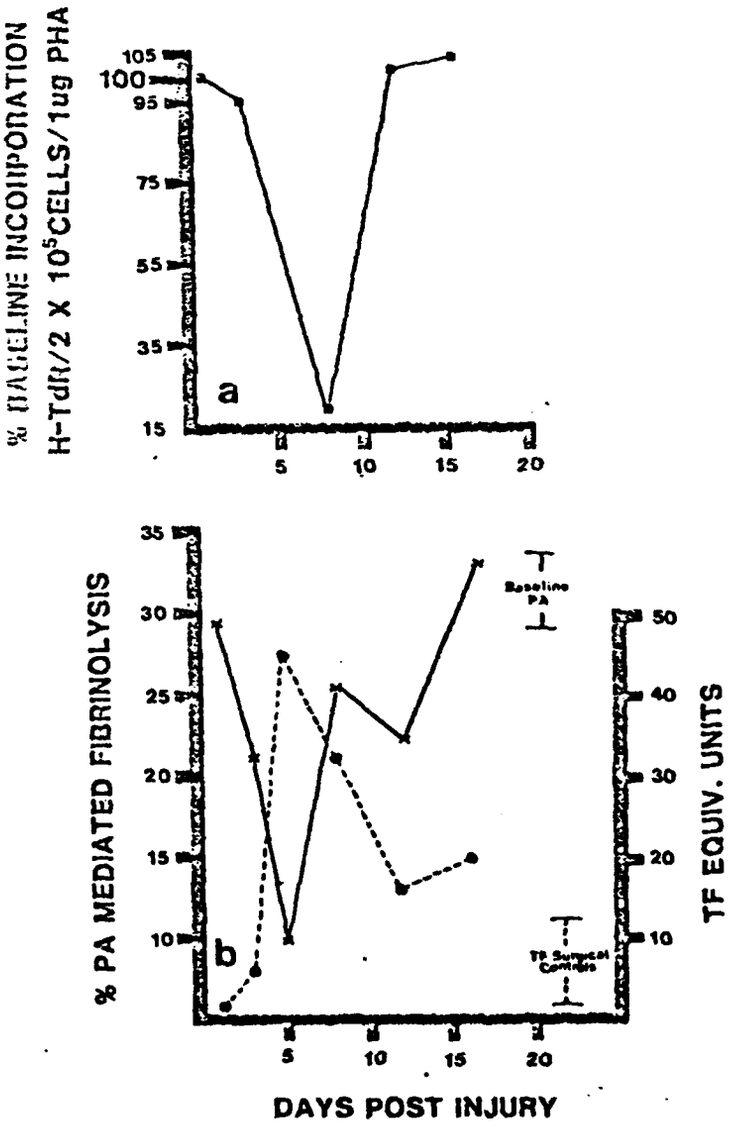


FIGURE 5

COMPARISON OF PHA HYPORESPONSIVENESS TO ALTERED MØ TF ACTIVITY IN A SEVERELY INJURED PATIENT

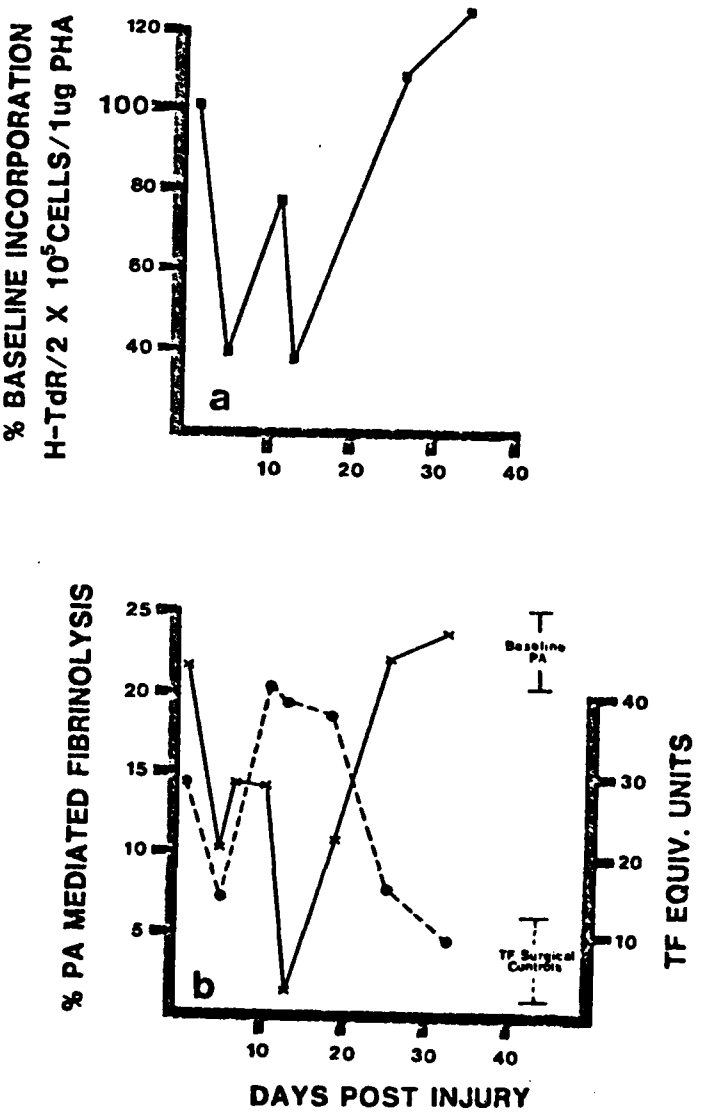
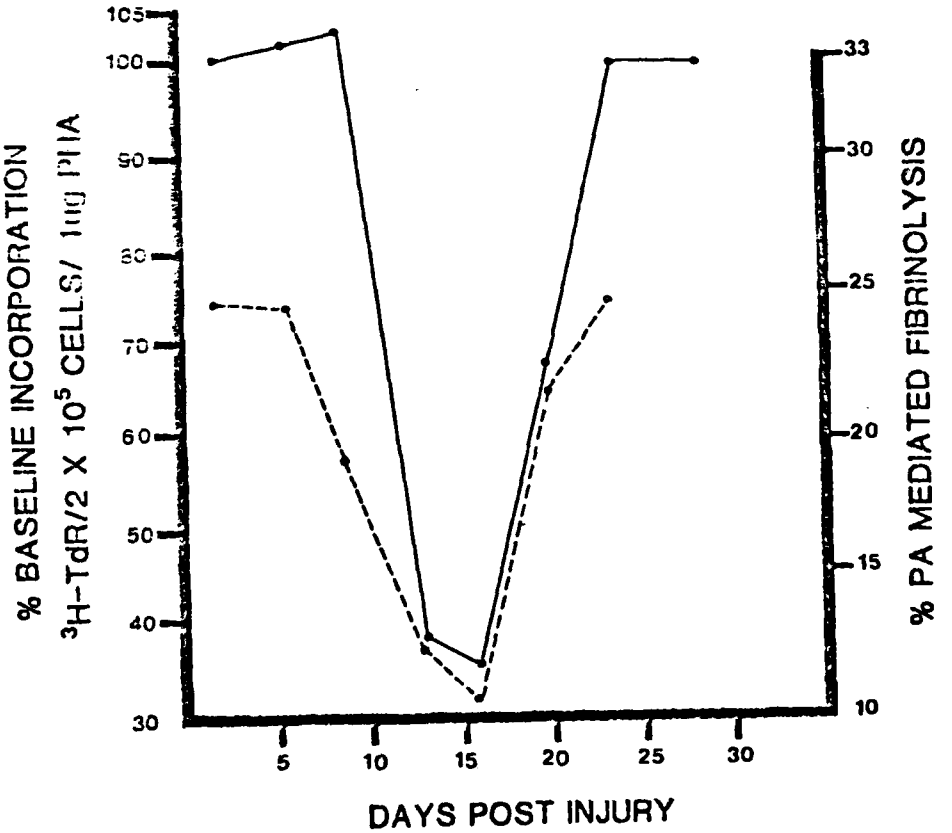


FIGURE 6

COMPARISON OF APPEARANCE OF DECREASED MØ PA FUNCTION TO DEVELOPMENT OF PHA HYPORESPONSIVENESS IN A SEVERELY INJURED PATIENT



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Recent Publications of Work Supported by this Contract:


1. Miller CL: 1981. Secondary Immunodeficiency in Burns and after splenectomy. In(Webster, A., ed.),Clinics in Immunology.

2. Miller CL, Linn D: 1980. T suppressor regulation of human monocyte function. Proceedings of IV International Congress of Immunology. Paris, France. July 21-26, (in press).

3. Graziano CJ, Miller CL, Lim R: 1980. The role of monocyte function in alteration of the thrombo-fibfinolytic system after shock. Surgical Forum XXXI (in press).

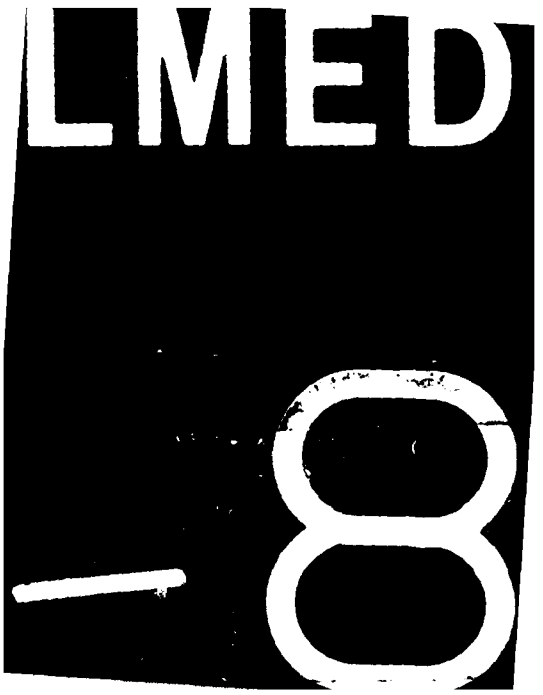
4. Miller CL: 1980. Alterations in macrophage function following thermal injury. In (Ninnemann J. L ., ed.). Immune Consequences of Thermal Injury. Williams and Wilkins, Maryland. (In press).

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


Submitted Papers:


1. Miller CL, Graziano CJ, Lim RC, Chin M: Production of tissue procoagulant factor by patient monocytes. Correlation to thromboembolic complication. Throm. Hemos.

2. Miller CL, Graziano CJ, Lim RC: Monocyte plasminogen activator production. Correlation to immune function. J. Immunology.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