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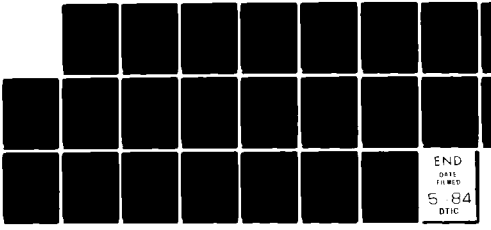

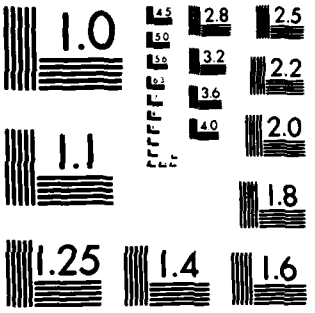

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STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY THERMAL INJURY

ANNUAL PROGRESS REPORT

Carol L. Miller, Ph.D.

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ARMY CONTRACT ANNUAL REPORT

Contract #DAMD 17-77-C-7012

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 heterogeneous 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 guinea pig 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). A complex recipro-

cal interaction occurs between facilitory MØ and T helper cells (1). Recent data indicates that a similar reciprocal interchange occurs between inhibitory MØ and suppressor T cells (2,3). We have spent this contract year devising and defining assays for measuring early MØ functions and determining 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). Monocytes from selected burn patients, who have been identified as being at high risk of septicemia, are analyzed for increased Prostaglandin E_2 (PGE_2) production using a radioimmunoassay (RIA). The post-burn appearance of increased suppressor cell activity is also monitored in the mitogen hyporesponsive burn patient. MØ plasminogen activator production has been suggested as paralleling MØ facilitory activity in murine systems (4,5). We have examined the correlation of human facilitory MØ activity to MØ PA production. Normal individuals' MØ produce only low levels of TF. High levels of MØ TF generation characterize the patient who is experiencing septic episodes or coagulopathy. Similarly, the patient whose MØ PGE_2 is augmented is immunodepressed. Appearance of excessive suppressor activity after thermal injury identifies a patient who will experience septicemia. Lysozyme synthesis is a general indicator of MØ viability and it appears to be unaffected by most specific inhibitory cells or molecules (6).

We have previously reported that there is an aberrant increase in inhibitory MØ after severe trauma and splenectomy (7,8). Any rise in inhibitory MØ (inh MØ) would severely depress immunocompetence since inh MØ prevent the proliferation of T helpers, B cells, and also interfere with facilitory MØ (fac 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 (9). 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 (10,11). Monocytes are removed by passing the mononuclear population over Sephadex G-10 columns (12). The B cells can be removed by nylon wool filtration of the cell population (13,14).

We monitor the ability of patient and normal mononuclear cell populations to respond to phytohemagglutinin (PHA) (15). This nonspecific mitogen response requires the cooperative interaction of monocytes and T cells (16,17).

Monocytes (MØ) are isolated from the Ficoll-Hypaque purified mononuclear cell populations by the Ackerman and Douglas rapid adherence technique (18). 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 into ^{125}I-fibrin plates and cultured 18 hours either in the presence of acid treated fetal bovine sera (AT-FBS) or soybean trypsin inhibitor (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 are 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 (4×10^5 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 (10). Human mononuclear cells are separated into T, B or MØ subpopulations and the interaction between these subpopulations is evaluated. T cells are segregated and isolated by formation of rosettes with SRBC (10,12). The effect of suppressor T cells or of inhibitory MØ is assessed by mixing purified autologous cell populations and assessing the affect of one cell type (i.e. suppressor T cell) on another cell population's function. The activity of inhibitory MØ is assessed by analyzing the increased production of PGE_2. We utilize a commercially available RIA kit which analyzes the PGE_2 content in MØ supernates by measuring the competitive binding of tritium (^3H) labeled prostaglandin and unlabeled sample PGE_2 with anti-prostaglandin antibody.

We have detected burn-induced aberrations in the immune regulation 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 is cultured in a "one way" MLR (15). In this assay, one group of the normal's cells are pretreated with mitomycin C (MC) to prevent their division (15). Consequently, this "one way" MLR assay measures the ability of one group's normal 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 measured in the MLR cultures.

A new assay for measuring burn-induced suppression has been developed by this laboratory. This assay assesses the ability of burn patients' E-rosette positive T cells and/or MØ to suppress PA production by a normal individuals' MØ. The burn-induced suppressive cells are incubated 24 hours with isolated normal controls' MØ, the normal MØ's are re-isolated, adjusted to 4×10^5/well and assayed in our PA system as described above. We compare the PA production of MØ incubated with allogeneic burn patients' cells to their PA production after exposure to allogeneic normal individuals' cells.

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 obtained from the no cell controls. The CPM's of ^{125}I-fibrin in the supernates from the lines containing cells in 100 μg SBI are subtracted from the CPM's of lines containing the cells in AT-FBS. This corrects for any ^{125}I-fibrin lysed by any non-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 ±8.4. The patient data were calculated by comparing the PA response at various days post injury to both the normal values (25 ±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. It has been suggested that human immune functions are controlled by immune response genes analogous to those described in animal systems [20,21]. 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 activities 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 coeffcient for determining the degree of interdependence between the various MØ and T cell parameters.

Guinea Pig Experiments.

Bilateral adrenalectomy (adx) is performed utilizing a modified Chevron subcostal incision. Right adrenalectomy is performed first, followed by left adrenalectomy. Following the surgery, the guinea pig (g.p.) is again given warm saline intraperitoneally at the rate of 30

cc/kg body weight. The g.p.'s are initially maintained on high dose cortisone at the rate of 15 mg/kg for 3 days and desoxycorticosterone at the rate of 6 mg/kg body weight for 3 days. After 3 days, maintenance doses of 7.5 mg/kg body weight of cortisone and 3 mg/kg body weight of desoxycorticosterone are utilized. At ten days post adx some of the animals are anaesthetized with Ketamine and receive a 30% scald burn (95^{o} C, 30 sec). At 3-4 days post-burn the animals are anaesthetized with Ketamine and a 15 ml blood sample taken. The g.p. are then sacrificed by CO_2 asphyxiation. Their spleens are sterilely removed for the AFC assay. The burned adx g.p. response is compared to that of normal g.p., burned g.p., and adx g.p.

The in vitro generation of AFC is assayed using the slide modification of the Hemolytic Plaque Assay (22). 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 (23). In order to augment MØ function, 2-mercaptoethanol (2ME) is sometimes added to cultures at a final concentration of 5×10^{-5} M.

RESULTS AND DISCUSSION

This contract year we have monitored post burn alterations in the host defense systems of nineteen severely burned patients. These nineteen severely burned individuals (>30% 3^{o}) were studied every 4 days post injury using our MØ and immune response assays. Of this severely burned group, 7 succumbed to overwhelming sepsis. We also continued to assess the trauma patients who had undergone splenectomy as a result of their injuries. We have assessed 14 asplenic trauma patients this year. This asplenic trauma patient group is known to have increased susceptibility to infection (24). Additionally, we have demonstrated that mononuclear cells from this asplenic trauma patient group develop suppressor T lympocytes (T_s), experience mitogen hyporesponsiveness, and manifest depressed MØ PA activity (Fig. 1). These same immune abberations (i.e., development of T_s, mitogen hyporesponsiveness, and decreased MØ PA activity) are also characteristic of the burn patient who develops overwhelming septicemia. However, there is a major difference between the kinetics of the immune hyporesponsiveness seen in burn patients and that exhibited by asplenic trauma patients.

The clinical correlation of MØ PA and mononuclear cell PHA hyporesponsiveness occurring in thermally injured patients seems to involve a slightly different syndrome. First, we have found that burn patients who show a decreased MØ PA response do not experience a concomitant inversely correlated increase in MØ TF production. This is in direct contrast to our results from asplenic trauma patients (2). Second, in the burn patient, decreased MØ PA function initially appears at 3-4 days post injury and peak depression is found at 5-9 days post injury. The maximal immune depression exhibited in the asplenic trauma patient, however, occurs 8-12 days post injury. Third, the severely thermally injured patient develops excessive suppressor T lymphocyte activity at 7-8 days post injury and these T lymphocytes can be shown to suppress MØ PA production. In contrast, asplenic trauma patients develop inhibitory MØ at 10-15 days post-injury. Additionally, MØ production of PGE_2 (as assessed in patient MØ supernates utilizing a radioimmunoassay) was found (in preliminary assays) to be 5 to 8 fold higher in PA hyporesponsive burn patients than in normal individuals or in other patient populations (Table 1).

Asplenic trauma patients who develop hyporesponsiveness do not

appear to experience elevated MØ PGE_2 levels. As previously discussed (2,3), we had originally suspected that increased PGE_2 production by inhibitory MØ might play a more important role in burn patients than in other trauma victims. Our preliminary PGE_2 data appear consistent with the supposition that increased MØ PGE_2 production may be a major contributor to the development of MØ PA and MNC PHA hyporesponsiveness in the burn victim. It is quite possible that the early increase in PGE_2 production by MØ seen in certain burn patients is a result of increased Ts and/or increased steroid (HC) levels. This possibility is being examined in our burn-adrenalectomized guinea pig model and is discussed below.

Not all patients with 30% 3^{o} burns develop MØ PA and MNC PHA hyporesponsiveness. Of the nineteen severely burned patients (>30% 3^{o}) studied, only seven succumbed to overwhelming septicemia. These seven patients also developed early and pronounced depression of their PHA and PA responses. A summary of the burn patients' responses is presented in Table 2. Coincident elevation of MØ TF was much more variable in this burn patient population (Table 2) than in the asplenic patients. As can be seen in Figures 2 and 3, the depression of the PHA and PA responses in these patients was early and pronounced while the elevation of the TF occurred later and was not reciprocal. Two burn patients had repeated septic episodes which were initially unresponsive to treatment, but eventually these patients recovered. The data from the two patients who experienced repeated septic episodes are illustrated in Figures 3 and 4. These patients' PA and PHA responses were depressed before their infectious challenges occurred. The patients were initially unable to properly respond to bacterial challenge with a hyperimmune response. Later in their recovery courses, however, these patients made the proper hyper-PHA response, as can be seen in Fig. 3. The TF values for these patients were massively increased only at a late post-burn period corresponding to their septic episodes. This may reflect the fact that both C split products (C3b and C5b) and endotoxin are known to increase MØ TF generation (25,26). One patient experienced a major pseudomonas infection but made an appropriate (hypernormal) response (Fig. 5). Interestingly, the TF values for this patient did not increase. There is obviously considerable variation in the effect of thermal injury on the burn patients' responses. However, those

patients who early in their clinical course experience PA and PHA hyporesponsiveness, appear to be unable to properly handle an infectious challenge that occurs during this depressed response period. Our MØ and immune assays do not predict which patients will experience an infectious episode, but only show how well the patient will contain a bacterial challenge when and if it does occur. It is also apparent that MØ PA, TF and the MNC PHA response are dramatically affected once an infection is in progress.

One of the most difficult problems with these assays is determining when the altered responses presage septic challenge and when these alterations in response are reflecting an ongoing bacterial infection. Our experience with these assays suggests that MØ PA and immune hyporesponsiveness which occurs within 3-5 days post thermal injury usually indicates a patient who will experience difficulty containing bacterial contamination. The normal individual and the burn patient with an unaltered host defense system respond to bacterial challenge with a hyperimmune response. However, as discussed in the contract proposal, some endotoxins and certain long lived C split products (generated as a result of infection) have been demonstrated as highly inhibitory to both immune and MØ PA responses-(27,28). Consequently, it becomes difficult to distinguish the cause of the continued depression of MØ PA and MNC PHA responses seen during infectious episodes. The measured hyporesponsiveness could have occurred because the patient was originally immunologically depressed due to injury and the added infection intensified that depression, or it is equally possible that the infection alone caused the continued reduction in the observed response. Clinical management of the patient also may affect his assayed immune responses. As can be seen in Figures 3 and 4, debridement and administration of antibiotics may influence assay findings. We are now attempting to test the effect of day-to-day clinical manipulation on the MØ PA and MNC PHA response.

As seen in Fig. 6, the data from the splenectomized trauma patients and that from the thermally injured patients are not identical. Clearly, MØ tissue procoagulant factor production and increased hypercoagulability are more important contributing factors in the asplenic patients' syndrome. In the burn patient increased TF production does not appear to play a major role, except perhaps in well

established bacterial infections. Consequently, we have attempted to develop additional assays for the burn patient which would not only identify early inimical alterations in host defense but also provide insight into the mechanisms which trigger these adverse alterations. We have previously utilized a three-way MLR to detect the development of excess suppressor cell activity (15). The drawbacks of this assay are that it has a six day incubation period, requires a well characterized and available panel of normal donors, is highly dependent on complex media (RPMI and Mishell-Dutton tested FBS), and must be incubated in special gas mixtures (7% O_2, 10% CO_2, and balance nitrogen). The results from our MLR system suggested the development of genetically unrestricted suppressor cells. These data have provided information about one pathway which leads to immunoincompetence after trauma. Obviously, the development of suppressor cells has a negative prognosis for the patients. However, the MLR system data is obtained too late to have any impact on patient treatment.

We are currently attempting to develop new assays which would detect excessive patient suppressor cell activity in a two day period. We have utilized the fact that both T_s and inh MØ will suppress fac MO function. Since the suppressive population that we originally described was not genetically restricted, we can analyze the ability of burn patients' MNC to suppress the PA response of normal individuals' MØ (Table 3). We have also investigated assays for MØ activities which would reflect different MØ immune functions. The suppressor cells which we originally detected in burn patients would non-specifically depress third party normal cells in our MLR system. The suppressive cells also rosetted with neuraminidase treated sheep erythrocytes (NSRBC). Consequently, we concluded that the suppressive cells were T_s. Unfortunately, recent data in the literature suggests that the E-rosette positive subset contains a large number of inhibitory MØ (29,31). It is possible, therefore, that the suppressive population consists of inhibitory MØ in addition to/or instead of T_s. There is also considerable new evidence in the literature suggesting that appearance of human T_s cells is always accompanied by simultaneous development of inhibitory MØ (23). As discussed in the contract renewal proposal, the interrelationship between these two types (T_s and inh MØ) may be so close that it is impossible to distinguish which activity appears first.

Both inhibitory MØ and T_s can suppress facilitory MØ activity, T_h function, and B cell antibody production (1,32). We have also produced data demonstrating that PGE_2 severely depresses MØ PA production while leaving lysozyme and TF production unaffected (Table 4). This is the same type of suppressive activity we see with purported T_s cells. Consequently, it is quite feasible that increased inhibitory MØ activity is at least a partial contributor to the post trauma depression of host defense systems we detect. We have attempted to use this ability of both inhibitory MØ and T_s to suppress facilitory MØ as a basis for a new and simpler suppressor assay which would detect both T_s and inhibitory MØ. In this assay, we add E-rosetting nonadherent cells (presumably mostly T cells) from patient PBL or Con A cultured normal PBL (control) to isolated MØ from a normal individual. First, 10^7 mononuclear cells are separated from peripheral blood using Ficoll-Hypaque densit, centrifugation. MØ are then isolated on Ackerman-Douglas flasks, yielding approximately 2×10^6 MO. 2×10^6 E-rosette positive cells are added to the MØ-containing flasks and incubated an additional 24 hours. The effects of adding patient cells to the MØ is compared to the result obtained following addition of allogeneic normal cells to the isolated monocytes. As can be seen in Table 5, addition of Con A-induced suppressor cells (see Methods, Page 9) significantly suppresses the MØ PA response as compared to control non-induced cells. The suppressor cells were E-rosette positive. In preliminary experiments, utilizing the suppression of the PA assay appears to be a quick method (results are obtained in 2 days) for detecting expanded suppressor cells in burn patient populations.

We have also analyzed the ability of patient MØ, T and B cells to cooperate in generation of specific antibody forming cells (AFC). Although this assay is an exquisitely sensitive experimental tool, it is totally impractical for patient screening. Even generation of a mitogen stimulated polyclonal antibody response requires specialized media, exacting culture conditions and a 6 day incubation period. Although the AFC assay remains an excellent method for detecting suppression and analyzing subtle cell interactions, it is not particularly useful in identifying high risk patients.

Our major experimental models continue to concentrate on dissecting the possible mechanisms by which thermal injury can trigger loss of host defense. As discussed in last year's application, we are currently examining whether a post-burn increase in steroid concentrations and PGE_2 production are major contributors to the decreased immune function seen in thermally-injured patients. Our primary experimental model is an adrenalectomized (Adx) inbred guinea pig. We have switched from the murine system to the guinea pig model for several reasons. First, the guinea pig is a larger inbred animal and adrenalectomy is therefore an easier procedure. Second, cortisol is the major glucocorticoid product in both guinea pigs and man (33). Third, the guinea pig and human complement systems are interchangeable (34). Fourth, a great deal of information is available on the components of the guinea pig immune system (34). Fifth, the methods for separating g.p. mononuclear cells are well established (35). Sixth, the g.p. AFC system is well defined, and the g.p. monocyte-T cell interaction is more similar to human cell interactions than those of murine systems (35). Additionally, immune induction in the g.p. system has been extensively characterized (1).

In preparation for the g.p. experiments, it was necessary to re-establish the method for inflicting a 20% 3^{o} burn in this species. We also needed to verify that a defect in the formation of antibody producing cells occurs at approximately 4-6 days post burn in these animals in order to correlate it with the time course seen in our murine and human systems. As can be seen in Table 6, the formulation of Ag specific AFC is maximally depressed in these animals at 5-6 days post burn. We had hoped to have more data in the g.p. system regarding the effect of adrenalectomizing the animals before thermal injury. If a burn-mediated increase in production of glucocorticoids is a major contributor to the immune hyporesponsiveness seen post-burn, then the immune responses of a burned adrenalectomized g.p. should be less depressed. In one experiment, comparing burned adrenalectomized animals to a burned sham-operated animal, there appeared to be less suppression of the AFC response. This very preliminary data is encouraging but far from definitive. Unfortunately, we have suffered a 3 month hiatus out of the 8 months since we initiated these experiments. The inbred g.p. population that we had, succumbed

to a salmonella infection which swept the entire g.p. colony at the animal facilities. The infection originated with animals from our local supplier and necessitated the destruction of the supplier's entire breeding population. The g.p. we ordered had to be shipped from the East Coast where there was a shortage of inbred g.p. We only recently have resumed our studies using the g.p. model. We are confident, however, that we can produce some exciting data in the next 4 months using the adx g.p. system to elucidate the role of the inh MØ in the augmentation of immune suppression taking place after burn injury.

One mechanism by which suppressive activity can be increased is to increase PGE_2 production by inh MØ (2-3). In addition, inh MØ are reported as having greater sensitivity to functional inactivation by carageenan than fac MØ. The fac MØ, on the other hand, has been shown to be differentially stimulated by dextran (36-38). Consequently, in vivo use of these compounds in our g.p. system can provide a means to dissect the mechanism of action of immune suppression mediated by MØ. We may be able to determine if increased inhibitory MØ activity, decreased facilitory MØ activity or both are involved in the augmented suppression seen after severe thermal insult. We have initiated experiments using in vivo administration of indomethacin, carageenan and dextran into adx g.p. in order to explore various distinct functional properties of inh MØ. The in vivo dosage of indomethacin and carageenan has been established for the g.p. system (3,36). These experiments are detailed in our new application. We intend to look at the combined effect increased PGE_2 and increased cortisol levels have in mediating post-burn immune depression.

Extensive investigations into a low molecular weight (13,000 daltons) substance extracted from burned murine skin were carried on by Dr. Peter Rist. Two materials were prepared. One was extracted from burned skin and one from sham injured control skin. These gift materials were assessed for their effect on in vitro generation of a primary AFC response, for their effect on MØ PA production and for their effect on MØ TF generation. As can be seen in Table 7, both extracts were equally depressive to the AFC and PA response while both elevated the TF levels. In the past no purported burn toxin had been simultaneously tested against a similarly prepared "control toxin" extract. These data strongly suggest that the lipid moieties

produced by the extraction technique are in themselves depressive and that this is not a result of the thermal injury. Additionally, the elevated TF response could indicate that these "toxin extracts" contain LPS (endotoxin) since endotoxin is known to both depress AFC and PA responses while increasing TF activity. After over 28 experiments, Dr. Rist found no evidence of a specific immunosuppressive burn toxin in the skin extracts. The data instead suggests that at least some of the immunosuppressive effects reported for burn toxin skin extracts are not the result of the burn injury to the skin. Rather the immunosuppressive effect may be due to extraction of normally present lipid moieties, to contamination of the preparation with endotoxin, or to both. Consequently, studies conducted on burn skin extracts which are not compared with normal skin extracts should be viewed in future with some skepticism. Dr. Shoenberger's large MW (300,000 daltons) material was also found by us to suppress the PA and AFC responses; however, since no "control toxin" material was available for comparison, the data is inconclusive.

The experiments in progress this year are designed to continue to monitor the various defects in host defense in the thermally injured patient. We have expanded our assays to attempt to detect T_s development in relation to inhibitory MØ appearance. Additionally, we are attempting to determine how various physiological changes (increased cortisol production, increased PGE_2 production) could mediate the post-burn development of immune abberations. Finally, we have initiated experiments examining the effect of interventive therapy designed to prevent or attenuate the development of adverse immune cell interaction after burns. The <u>in vivo</u> use of indomethacin, carageenan, and dextran in our g.p. model should provide information not only on the mode of action of burn-mediated immune depression but also on possible prophylactic treatments.

TABLE I

Increased PGE_2 production[1] by MØ from severely burned patients

	Exp 1	Exp 2	Exp 3	Exp 4
Patient	1500	975	3500	3000
Control[2]	300	300	580	328

1. PGE_2 in picograms per sample of 10^7 MØ.

2. Normal individual run in same assay.

Table II

Correlation of Burn Patients' Immunologic Parameters to Clinical Course

Patient	Maximum % PA Suppression[1]	Maximum % PHA Variation[2]	Outcome
		Group I	
PM	15	+20	No complications - Released
FF	26	-18	No complications - Released
FM	21	-15	No complications - Released
SM	22	+25	No complications - Released
WM	20	+30	No complications - Released
HM	24	+40	No complications - Released
PF	15	-12	No complications - Released
		Group II	
CF	35	+220	Pseudomonis infection - Recovered
GM	20	+260	Pseudomonis infection - Recovered
KM	23	+200	DVT - Recovered
MM	30	+185	Staph. infection - Recovered
HM	33	+300	Staph. infection - Recovered
WM	21	+450	Pseudomonis infection - Recovered
		Group III	
MF	67	-70	Succumbed to Staph. Sepsis
CM	82	-80	Succumbed to Serratia Sepsis
HM	83	-71	Succumbed to Pseudomonias
DM	62	-80	Succumbed to Pseudomonias
RF	62	-65	Succumbed to Enterococci
TF	65	-85	Succumbed to Staph. Sepsis
SM	80	-72	Succumbed to Serratia Sepsis
LM	61	-60	Pseudomonias, Staph.-Eventually recovered
MM	70	-60	Staph., Serratia, Enteroccus, Septisemia-Eventually recovered

1. Maximal percent of suppression that occured during post injury period.

2. Day of maximal variation in PHA response from baseline does not coincide with day of maximal PA suppression except for Group III.

Table III

SUPPRESSION OF HUMAN Mϕ PA PRODUCTION BY INCUBATION OF Mϕ WITH SUPRESSOR T CELLS[1]

Suppression of PA response of 4 x 10^5 Mϕ previously incubated with 4 x 10^5 cell

Source of "suppressive" cells	Exp A	Exp B	Exp C
Autologous Culture T	2.4	-6.8	-8.3
Autologous Culture Mϕ[2]	-1.8	12.6	-4.4
Autologous Suppressor T	70.3	72.4	89.5
Autologous Suppressor Mϕ	4.7	31.0	5.1

	Exp D	Exp E
Allogeneic Culture T	11.7	6.2
Allogeneic Suppressor T	40.7	55.2

1. T cell population derived from E-rosette positive population cultured 24 hrs either with or without 1.5ug Con A.

2. Mϕ population derived from adhered E-rosette negative population.

TABLE IV

Suppression of MØ PA production by 5×10^{-6} M PGE_2

4×10^5 MØ/Culture	% Suppression[1]		
	Exp A	Exp B	Exp C
MØ + PGE_2	64	46	46
MØ + Indo[2]	-14	8	-13

1. Percent suppression as compared to control response.

2. Cells cultured with 5×10^{-6} M Indomethacin.

TABLE V

Change in MØ Function After Addition of Suppressor Cells

% Suppression[1] PA	% Suppression TF	% Suppression Lysozyme
65	-50	-84
68	-38	-89
66	-41	-50
67	-45	-107
68	-43	-61

1. Percent suppression as compared to control response.

TABLE VI

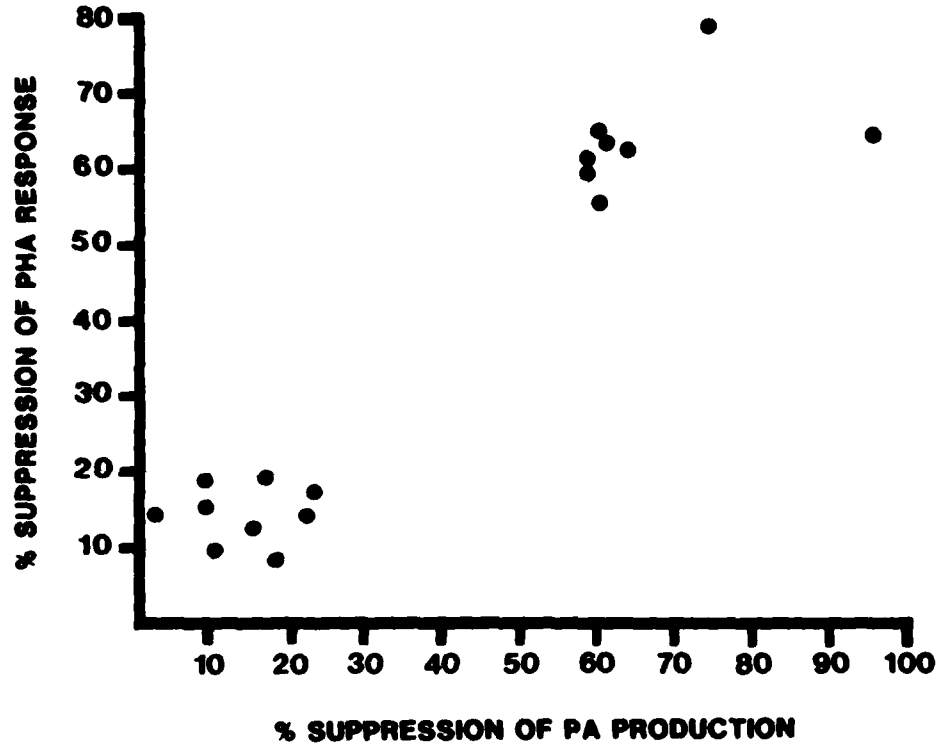
Depressed AFC Response in Burned Guinea Pigs

Animal Used	Number of AFC Generated to SRBC[1]		
	Exp A	Exp B	Exp C
Burned[2]	6	0	0
Sham	177	150	64

1. Number of Antibody Forming Cells (AFC) generated in response to in vitro challenge with sheep erythrocytes.

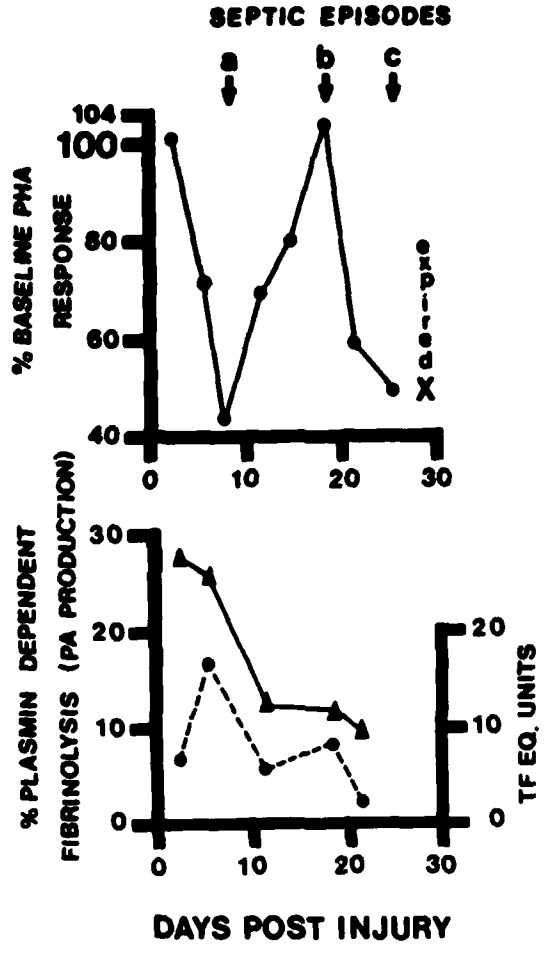
2. Guinea pigs with 30% 3^{o} burn sacrificed on day 5 post injury.

FIGURE 1



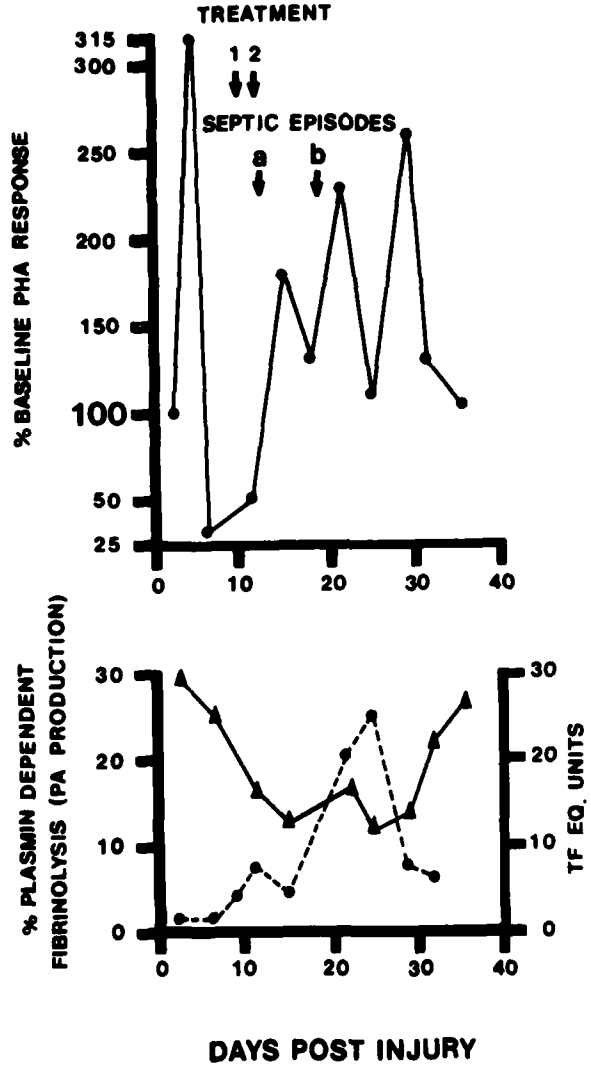
CORRELATION BETWEEN MITOGEN AND HYPORESPONSIVENESS AND DEPRESSED MØ PA ACTIVITY

FIGURE 2



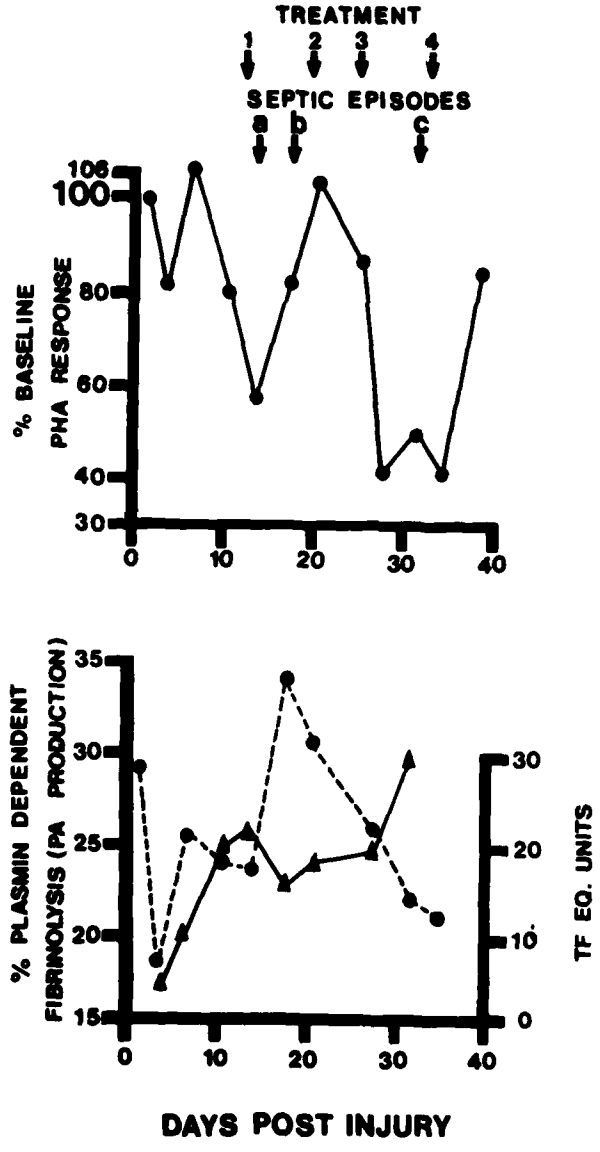
POST-INJURY CHANGES IN LYMPHOCYTE AND MONOCYTE ACTIVITIES AFTER SEVERE THERMAL INJURY.

FIGURE 3



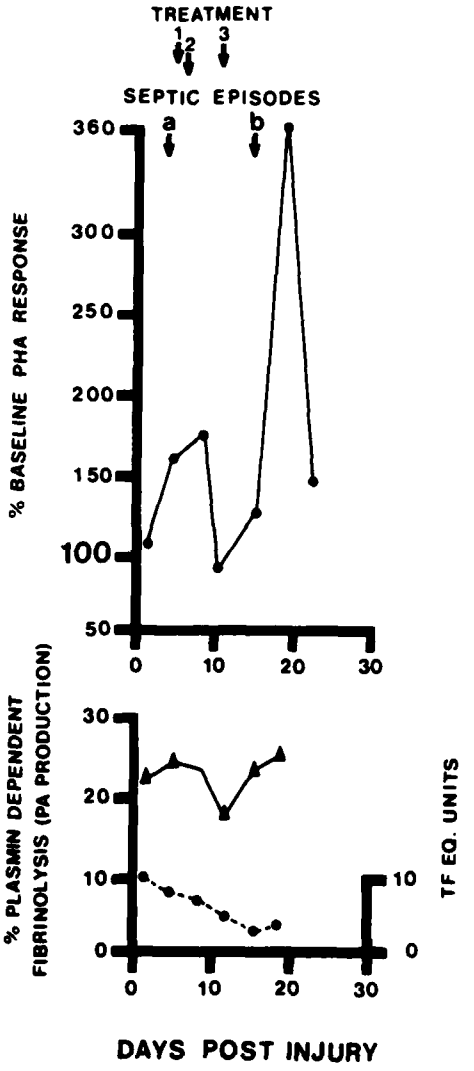
DEPRESSION OF PHA RESPONSES AND PA RESPONSES IN RELATION TO THE OF SEPSIS IN A BURN PATIENT

FIGURE 4



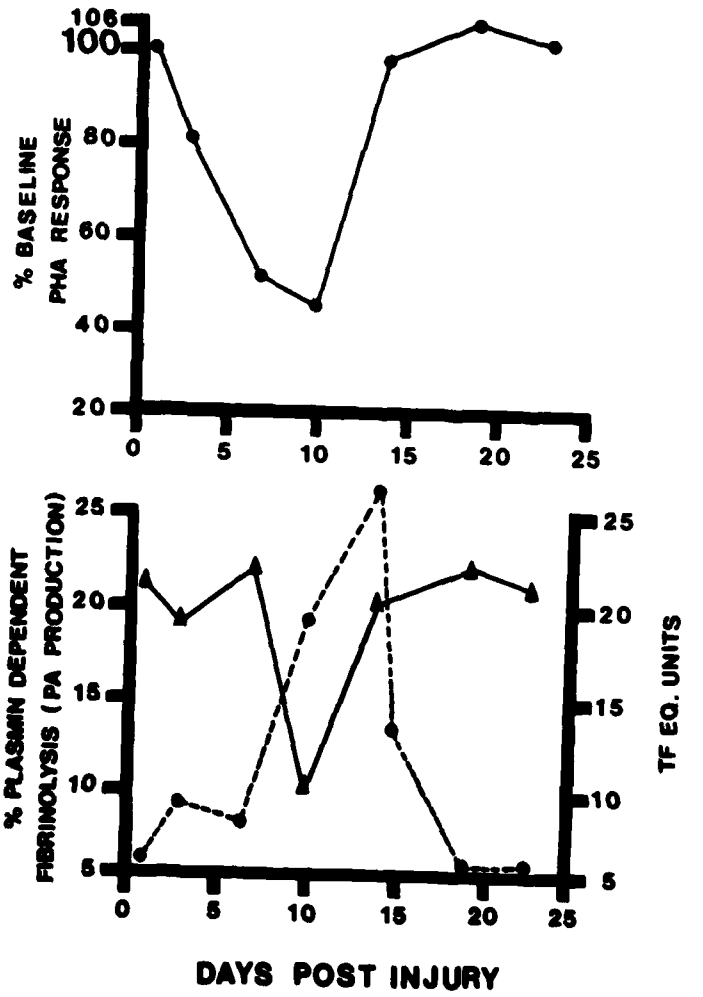
DEPRESSION OF PHA AND PA RESPONSES IN RELATION TO THE ONSET OF SETSIS IN A BURN PATIENT

FIGURE 5



PHA, PA AND TF DATA FROM A BURN PATIENT WHO EXPERIENCED TWO INFECTIOUS EPISODES

FIGURE 6



COMPARISON OF PHA, PA, AND TF RESPONSES IN AN ASPLENIC TRAUMA PATIENT WITH MULTIPLE INJURIES

Figure 4 - PHA, PA, and TF data from a thermally injured patient with recurrent septic complications.

Upper graph illustrates the alterations in mitogen response. Arrow under septic episodes indicate initial isolation of organism from patient cultures (a = enterococcus; b = Staph. aureus; c = strep). Arrows under treatment indicate major clinical manipulation. Each time a different antibiotic was started, it is indicated on graph (1 = antibiotics; 2 = antibiotics; 3 = antibiotics; 4 = antibiotics). Lower graph illustrates concomitant PA (solid line) and TF (broken line) data.

Figure 5 - PHA, PA and TF data from a burn patient who experienced two infectious episodes but made appropriate responses.

Arrows under septic episodes indicate organisms isolated (a = strep.; b = candida). Arrows under clinical management indicate major manipulations (1 = antibiotics; 2 = debridement; 3 = antibiotics). Lower graph illustrates MØ PA and TF responses at post injury days indicated.

Figure 6 - Comparison of PHA, MØ PA, and MØ TF response in an asplenic trauma patient with multiple injuries.

Upper graph illustrates the percent of baseline ^{3}H-TdR incorporation by a patient's mononuclear cells. Lower graph illustrates the alterations in the MØ PA (solid line) and MØ TF (broken line) over the same post injury time course. Mononuclear cells and MØ were isolated from the same sample. MØ PA data are presented as percent of plasmin mediated fibrinolysis while MØ TF data are given as number of equivalent units (TF Eq. Units). The equivalent units are derived from a rabbit brain thromboplastin standard.

LEGEND

Figure 1 - Correlation between mitogen hyporesponsiveness and depressed MØ PA activity.

The maximal depression of the PHA response seen during the post injury period is plotted against the maximal depression of MØ PA activity. The post injury day on which this minimum response observed was not always identified.

Figure 2 - Comparison of mitogen response data and MØ PA and TF data from a thermally injured patient who succumbed to septicemia.

Upper graph illustrates the PHA data. Post injury day of initial isolation of organisms from patient cultures is indicated with arrows, and a = Staph. aureus; b = candida; c = pseudomonas septicemia. Patient expired with pseudomonas septicemia.

Figure 3 - Comparison of PHA, PA, and TF data from a thermally injured patient who experienced recurrent septic episodes.

Upper graph illustrates alterations in the mitogen response. Lower graph represents data on MØ PA (solid line) and TF (broken line). Arrow under septic episodes indicate isolation of positive cultures (a = pseudomonas; b = Staph. aureus). Arrows under treatment indicate major manipulation (1 = debridement; 2 = antibiotics started). Lower graph illustrates MØ PA and TF values on various day post injury.

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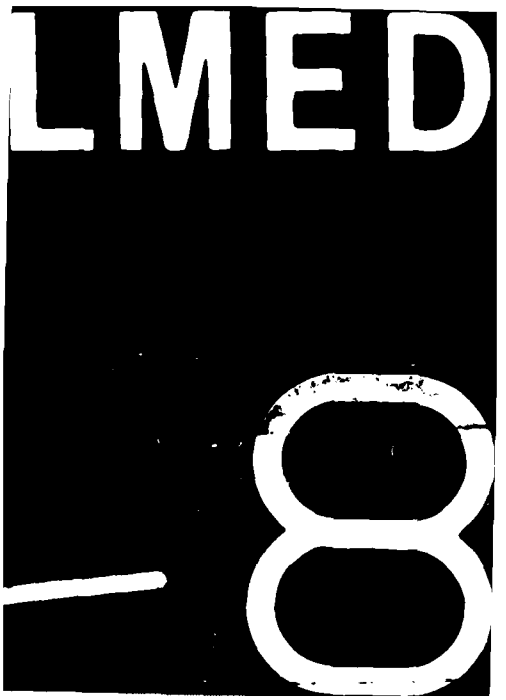
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