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STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY THERMAL INJ--ETC(U)
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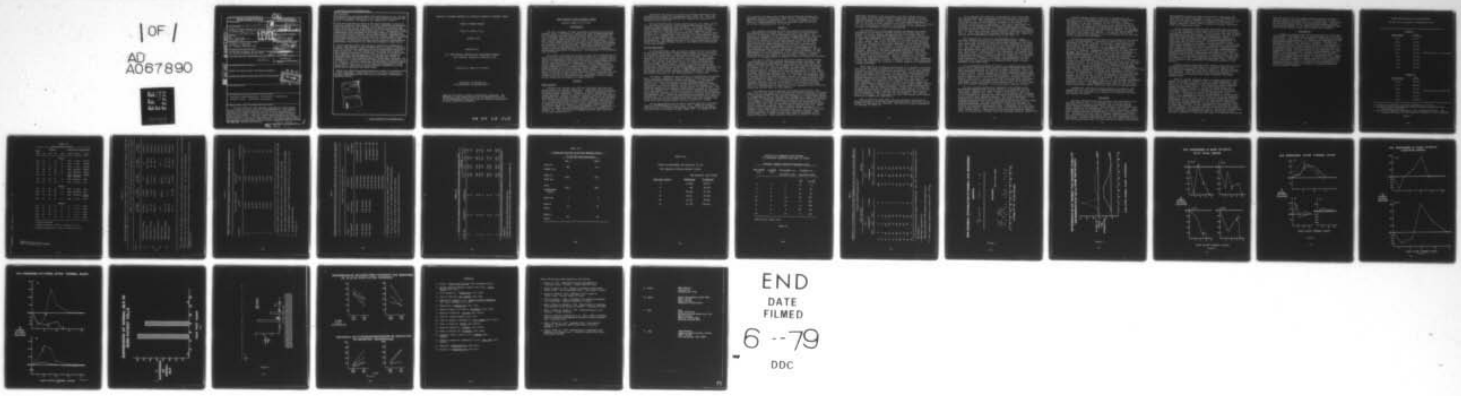

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The high incidence of fatal septicemia associated with severe thermal injury is believed to result from loss of immunocompetence. This laboratory has been able to identify those burn patients who are at greatest risk for developing fatal sepsis by 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 (cont)

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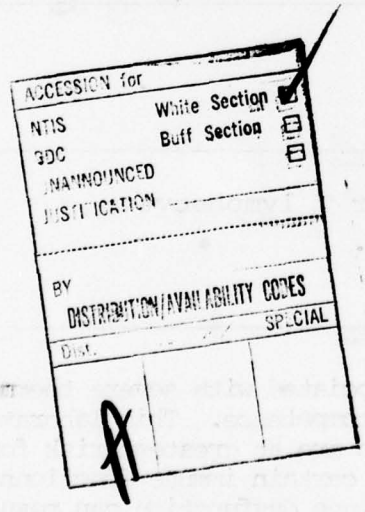
subpopulations are the antigen specific bone marrow-derived (B) cell, the antigen specific thymus-derived (T) cell, and a third extremely heterogenous population of leukocytes - the monocyte or the accessory cell (A cell).

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 most pertinent results from this year's research can be summarized as follows: Those thermally injured individuals who will be unable to contain an infectious challenge have leukocytes which are PHA hyporesponsive beginning at days 4-6 post burn. All other thermally injured patients have unchanged or hyper-responsive leukocytes in the PHA assay. 85 percent of the burn patients whose lymphocytes were hyporesponsive in the PHA assay later succumbed to septicemia. These same PHA hyporesponsive leukocytes would suppress the allogenic specific mixed lymphocyte response of normal human leukocytes. The suppressive cell in these burn patients' leukocyte population was identified as a T lymphocyte. These data indicate that the development of excessive suppressor T lymphocyte activity is one of the pathological consequences of severe thermal injury. Additionally, excessive inhibitory macrophage activity was demonstrated as developing after severe trauma.

Whether this inhibitory macrophage develops as a result of T suppressor action is being investigated. Characterization of the cell types involved in the decreased immunocompetence seen after burns is preliminary to development of appropriate therapy.



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 the accessory cell (A cells).

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.

Methods

Human studies

Patients with greater than 20% 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 by Ficoll-Hypaque gradient centrifugation (1). 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 (2). Monocytes are removed by passing the mononuclear population over Sephadex G-10 columns (3). The B cells can be removed by nylon wool filtration of the cell population (4).

We monitor the ability of patient and normal mononuclear cell populations to respond to phytohemagglutinin (PHA) (5). This non-specific mitogen response requires the cooperative interaction of monocytes and T cells (6).

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 (7). In this assay one of the normal's cells are pretreated with mitomycin C (MC) to prevent their division (5). Consequently, this "one way" MLR assay measures the ability of one normal's 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.

Murine experiments

In each experiment, BDF_1 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 metafane anesthesia, littermate mice are shaven and then divided in two groups. One group receives a 10-20% scald burn with 95° 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 (8). 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.

"A" 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 A or T cells are supplied to the immunodepressed burned mice's cells. These experiments examine whether supplying normal, functional A or T cells restore the ability of the thermally injured leukocyte population to generate normal numbers of specific AFC. "A" cells are depleted from leukocyte populations according to the method of Ly and Mishell (3). T cells are lytically removed from leukocyte populations by treatment of the splenocytes with anti-T cells antisera and complement (9). Leukocyte populations are depleted of B cells by irradiation. "B" cells can also be depleted from Splenocyte populations by passing the cell preparations over nylon wool columns (10). These nylon wool columns remove A cells, as well as B cells.

The in vitro generation of AFC is assayed using the slide modification of the Hemolytic Plaque Assay (11). 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 condition media is produced as described (12). In order to augment A cell function, 2-mercaptoethanol (2ME) is sometimes added to cultures at a final concentration of 5×10^{-5} M.

Results

The research supported by this army contract has resulted in the development of a simple assay for detection of those patients which are at greatest risk of developing fatal septicemia. An increase in suppressor T cell activity was demonstrated as resulting from thermal injury in our murine model. These data lead to the design of a modification of the MLR response to detect suppressor cell activity in burn patients' mononuclear cells. We not only demonstrated that human suppressor cell activity increased after burns, but also that the suppressive cell was in fact a T cell. The mechanism of burn mediated reduction of immunocompetence has been investigated both in our murine model and in patients. We have demonstrated that a macrophage (Ø) defect occurs concomitant to the development of T cell suppressors in our murine model. A similar Ø defect appears to be present in burn patients. Finally, we have developed an in vitro system to measure a specific primary AFC response.

The need to develop a simple monitoring system for burn patient immune capacity was approached by examining mitogen responses. Previous investigators had published data showing that the average PHA response of burn patients was increased over the average PHA response of normals (13, 14). When we examined the individual PHA responses of burn patients at various times after thermal injury, we obtained divergent results. Examination of the PHA responses of a large number of normal individuals revealed that the population did not have a normally distributed PHA response (Fig. 1). Averaging PHA responses tends to obscure all but the most dramatic changes. Any one individual, however, repeatedly tested in our system, has a PHA response which varies not more than 11% (Table I). Our initial study of burn patients' PHA responses revealed that the PHA response of all surviving patients studied eventually returned to the initial 1-2 day post burn level (Fig. 2). Consequently, we used this 1-2 day response as each patient's normal PHA control level or 100% response.

In studies on 23 burn patients with acute thermal injury (see Table II), we have found a 100% correlation between reduction of patients' cells' mitogen responsiveness and development of life threatening sepsis. As illustrated in Fig. 3, all patients succumbing to fatal sepsis showed a markedly decreased PHA response that preceded any evidence of sepsis by 4 to 6 days. In striking contrast, mononuclear cells from patients who had infectious episodes, but no severe sepsis, had increased PHA responses (Fig. 4a). The two patients who survived severe sepsis showed first a decreased response, then a dramatic increase (Fig. 5). Those patients with a sepsis free clinical source showed essentially no change in their PHA response over time (Fig. 4c). The arrows on these figures indicate the onset of clinically detectable sepsis. These data suggest that a simple mitogen test could be performed at far forward locations. Those patients identified as unable to contain

infectious challenge (≈30% of patient >30% third degree burned in this study) could be segregated and immediately removed to a more complete hospital facility. Those patients not requiring such care could be treated at field hospitals. The PHA hyporesponsiveness seen in the group of burn patients could result from a defect in Ø and/or T cell proliferative function or an increase in the regulatory activity of suppressor T cells or inhibitor Ø.

The availability of more sophisticated techniques and genetic information in the murine antibody forming cell system (AFC) has allowed us to more closely characterize the burn induced immune defect. As we have previously demonstrated, a 20% scald burn severely depresses development of a de novo antibody forming cell response in mice. This defect is not the result of a bursal equivalent cell (B cell) defect. The B cells from burned mice can produce antibody normally in cooperation with normal syngeneic T cells and macrophage. As the data in Table III illustrate, the addition of syngeneic splenocytes from burn mice, to cultures of normal splenocytes will prevent the normal cells from generating a de novo antibody forming cell response. Addition of the same numbers of splenocytes from sham injured animals augments the numbers of AFC generated. The T cells were depleted from the burn mouse splenic population by treatment with anti-θ sera and complement. Specific removal of the T cells removed the suppressive activity. These data indicate that suppressor T cells are generated as a result of thermal injury. These suppressor T cells then prevent the induction of immune responses to subsequently invading organisms.

These results in the murine system lead us to develop a modification of the MLR which is specifically adapted to detection of suppressive cells in patients' mononuclear populations. In our modified MLR systems, we employ reduction of a "one way" MLR response between two normal individuals as an indicator of suppression. Peripheral blood mononuclear cells from normal volunteers were isolated by Ficoll-Hypaque sedimentation as described under Methods. One group of normal's cells were mitomycin C (MC) treated to prevent their cells from responding. A second group of normal's cells were assayed for their proliferative response to a variety of MC treated normal stimulators' cells. Twelve highly responsive "one way" normal combinations were selected from the normals screened. Mononuclear cells from burn patients were collected at 3-day intervals after injury. The ability of these patient cells to suppress the 3H-TdR incorporation of the highly responsive normal "one way" MLR was assessed. Cell numbers were kept constant. We were aware that this test system would usually detect only nonspecific suppression.

Only in the rare cases where the burn patient's cells and the normal responder's cells were histocompatible would both specific and nonspecific depression be detected. The results of these experiments are depicted in Table IV.

It is apparent that addition of mononuclear cells from those burn patients who were hyporesponsive to PHA severely suppressed the normals' MLR. This suppressive activity of the burn patient's cells was not due to cell crowding, nonspecific cell death, or allogeneic stimulation of suppression. Only cells from those burn patients who had depressed PHA responses exhibited suppressive activity in this system (Table V). Normal individuals, or moderately burned patients' cells did not significantly suppress the normals' MLR. In fact, these severely burned patients' cells were suppressive only at a time when their PHA response was depressed (≈5-7 days post injury) and not immediately after their injury (Fig. 7).

The identity (Ø, T or B) of the suppressive cell in burn patients' leukocyte populations is being investigated. Removal of the T cells from the burn patients mononuclear populations (by rosetting techniques) resulted in reduction of their suppressive activity (Fig. 8). These data implicate the T cell population as the suppressor. However, there is suggestive evidence from our MLR system that two suppressive cells may be generated after burns. This evidence is that the Mitomycin C sensitivity of the suppressor cell changes with time (Table VII). The suppressor T cell previously reported in human leukocyte populations is usually Mitomycin C sensitive. In contrast, Ø inhibitors are Mitomycin C resistant. The mechanism(s) by which these suppressive T cells act to reduce immunocompetence and the mechanism which causes them to be generated after burns is being examined in our mouse model.

In a new murine experimental design, we investigated the possibility that T suppressor cells depress general immunocompetence after burns by interfering with Ø function in immune induction. This is a well established pathway for some artificially stimulated murine suppressor T cells. Such a pathway has not been previously demonstrated as occurring as a result of pathology, however. In preliminary experiments it appears that Ø activity is decreased in thermally injured animals (Table VIII). If the murine model indicates that a Ø dysfunction results from T suppressor action, we will initiate Ø depletion experiments using burn patients' mononuclear cells. It is very likely that burn induced suppressor T cells can affect monocyte activity.

We have already identified an inhibitory Ø as mediating the mitogen hyporesponsiveness we have defined in splenectomy patients (Fig. 9ab). Depletion of these Ø allows the patients' T cells to proliferate normally (Fig. 9cd). There is also considerable evidence in the literature to indicate that inhibitory monocytes act as regulators of normal human immune responses. The current hypothesis is that unbalancing of the proportions of these monocytes in the total leukocyte populations can result from patient pathology and cause immune hyporesponsiveness. The same experimental design we have used to examine splenectomy patients' Ø activity is now being initiated for testing burn patients' mononuclear cells. Preliminary data from this type of experimental design indicate that an inhibitory Ø is generated after thermal injury. Depletion of the Ø from burn patients' mononuclear cell populations reverses their PHA hyporesponsiveness (Table VIII).

Another immune function which can be assessed in burn patients is the development of specific antibody forming cells. The monitoring of the development of AFC provides a direct measurement of the burn patients' ability to resist infection. The kinetics and advent of depression of AFC generation can be compared with the appearance of suppressive activity for the MLR and the onset of depression of the mitogen response. The in vitro generation of specific AFC appears to be an efficacious method to evaluate human leukocytes function. It also was particularly suited to separately examining A cell facilitation due to the kind of AFC system we have developed.

We are still attempting to improve our methods for monitoring patient immunocompetence. Inhibitory Ø appear primarily in the peripheral blood (PB) leukocyte population. These inhibitory Ø occur even in normal human PB. It is these inhibitory Ø which have hampered the development of an in vitro measurement of patients' ability to generate a primary specific antibody forming cells (AFC) response. Assessment of the ability to generate a de novo, specific AFC response represents the best measurement of patient immunocompetence. We have been utilizing Ø purified from bronchial washouts combined with autologous Ø depleted peripheral blood lymphocytes to generate specific AFC responses (Table IX). The drawbacks to this system are that it is limited to intubated patients and requires instilling some fluid into patients' pleural cavities. We are examining an additional approach for circumventing the action of inhibitory Ø in generation of in vitro specific AFC. Recent experiments reported in the literature have indicated that inhibitory Ø regulate by secretion of prostaglandin E_{1-2} (15). Several reports of reversing this inhibitory Ø effect by addition of indomethacin (a prostaglandin inhibitor) have been published. We reasoned that it might be possible to incorporate indomethacin into the PB cell cultures at micromolar concentrations, thereby reversing the inhibitory Ø effects. The results of such experiments are illustrated in Table X. As can be seen, indomethacin inclusion allows the in vitro generation of specific AFC from PB mononuclear cells. The indomethacin alone does not act as a polyclonal stimulator causing nonspecific secretion of immunoglobulin.

Indomethacin containing cultures without Ag produce no AFC. Consequently, we feel that the indomethacin system holds great promise for the development of a specific AFC response utilizing only patient PB cells.

Discussion

The data obtained during this contract year established that the burn patient, who is at increased risk of sepsis, can be identified by detecting a progressive loss of his mononuclear cells' mitogen responsiveness. These patients' cells develop this immune aberration at approximately 4 to 6 days post injury. Those burn patients who will subsequently be unable to contain an infectious challenge showed decreased ability to generate a response to PHA. Concomitant to their loss of mitogen responsiveness, these burn patients develop suppressive cells in their mononuclear cell population. Whether these suppressor cells are responsible for the PHA hyporesponsiveness seen in these patients is being investigated.

The suppressive cells in these burn patients' leukocytes were identified as being suppressor T cells. It is of interest to note that the time of appearance of immune depression in the burn patient (4-5 days post injury) is identical to the onset of immune dysfunction (loss of AFC response) previously identified using our burned mouse model.

In our murine burn model we have determined that suppressor T cells are generated in the burned mice at 4-5 days post injury. It is these suppressor T cells which are causing the loss of immunocompetence in the burned mice. Significantly, the burn patients also were demonstrated as developing suppressor T cells. It would seem highly likely that these burn induced suppressor T cells are also responsible for the increased susceptibility to infections seen in burn patients. We have developed a test system which enables us to examine the generation of specific AFC using human peripheral blood cells. Employing this new human AFC test system, we should be able to definitively investigate how burn induced suppressor T cells affect patient immunocompetence. The indomethacin system may provide a new means of accurately assessing any patients' immunocompetence after injury. The indomethacin system requires only 10 ml of peripheral blood from the patients. It is much more adaptable to experiments investigating the kinetics of loss of immunocompetence after burns. However, the BW system is more applicable to dissecting possible A cell defects.

We have already established that certain trauma patients show a progressive loss of their ability to generate specific AFC. Utilizing our in vitro AFC system, we expect to demonstrate that burn patients' cells also lose their ability to generate specific AFC responses. Most importantly, our AFC system appears to identify those patients who are likely candidates for septic complications.

There is another important aspect of these experiments which examine the effect of thermal injury on immunocompetence. This aspect is the determination of how burns cause immune anomalies. Utilizing our murine model we have uncovered a loss of monocyte immune function after burns. It has been demonstrated that adequate monocyte function is crucial to proper initiation of the immune response (16). In the absence of proper Ø activity, suppressor T cells are stimulated in preference to helper T cells (17). Consequently, one of the mechanisms by which thermal injury could unbalance the immune system, would be via depressing monocyte (A cell) function. We are currently analyzing monocyte functionally in the burn patients. We have already shown that burned mice develop suppressor T cells and have a Ø dysfunction. We have also demonstrated that burn patients have aberrant levels of suppressor T cells. We are attempting to establish that burn patients develop a progressive monocyte dysfunction which precedes their development of suppressive T cells. We are also investigating the cause-effect relationships between the appearance of burn patients' suppressor T cells and the onset of a monocyte dysfunction.

Identification of all the immune cellular defects occurring in the burn patient and delineation of the onset time of these defects permits initiation of proper immunotherapy. Understanding the mechanisms by which burns compromise immunocompetence may allow development of far forward prophylactic measures which would prevent thermal injury from inducing such defects.

Conclusions

A subset of patients with 30% third degree burns have been demonstrated as developing PHA hyporesponsiveness at 4 to 6 days post injury. This subset of burn patients subsequently developed severe septic complications. It appears that development of PHA hyporesponsiveness can be used as an indicator of those burn patients at greatest risk of septic mortality. Additionally, it was shown that severe thermal injury caused the development of T suppressor cells in both a controlled, burn murine model and in patient groups. The significance of the appearance of these suppressor T cells to loss of patient immunocompetence was discussed. The development of a monocyte defect after thermal insult was established as occurring in our murine model. Abrogation of monocyte function results in unbalancing of the immune network and predisposing the immune response toward regulation (i.e. suppressor T cells). Consequently, it is suggested that thermal injury may result in a loss of immunocompetence and increased susceptibility to infection by causing progressive loss of monocyte function.

RESTRICTED VARIATION OF PHA RESPONSE

FOR ANY ONE INDIVIDUAL'S MONONUCLEAR CELLS[a]

Normal I		
Date tested	CPM[b]	
10/2	61,777	
10/18	79,895	
10/31	72,395	
11/15	68,283	
12/6	56,693	$\bar{X}$ CPM[c] 67,335 $\pm$ 6,768 (10%)
1/6	71,008	
1/17	67,764	
1/23	62,801	
2/10	65,396	

Normal II		
Date tested	CPM	
10/13	98,695	
11/4	101,866	
11/22	85,261	$\bar{X}$ CPM 92,235 $\pm$ 8,306 (9%)
1/12	97,823	
2/6	88,237	
2/13	81,530	

a Average of triplicate cultures' response to 1 ug PHA.

b Change in CPM of incorporated H-TdR in mitogen stimulated minus unstimulated cultures.

c Mean of all separate experiments $\pm$ one standard deviation. The standard deviation is also given as a percent of the $\bar{X}$ response.

TABLE I

Table II

Clinical Data on Burn Patients

Patient & Sex	Age	Area Burn 2°	Area Burn 3°	Lung†	Clinical Course (Post Burn Day)* Sepsis¶	Organism	Outcome
				Group I			
1-M	45	--	60	S	S(7)	S. aureus	Died(8)
2-M	27	--	45	S	S(21)	E. coli	Died(29)
3-M	37	10	45	N	S(12)	E. coli	Died(23)
4-F	70	10	35	S	S(10)	Pseudomonas	Died(12)
5-F	20	--	65	N	S(12)	Klebsiella	Died(14)
6-M	66	20	15	S	S(15) S(42)	Enterococci Pseudomonas	Died(45)
7-M	58	10	45	N	S(15)	Klebsiella	Lived
8-M	27	--	80	M	S(8)	S. aureus	Lived
				Group II			
9-M	29	65	10	M	M(15)	S. aureus	Lived
10-M	35	20	20	S	M(18)	Enterobacter	Lived
11-M	76	20	10	S	M(20)	Klebsiella	Died of MI (85)
12-M	43	15	15	S	M(15)	E. coli	Lived
				Group III			
13-M	24	11	35	S	N	---------	Lived
14-F	45	28	5	N	N	---------	Lived
15-F	42	10	20	N	N	---------	Lived
16-M	21	20	5	N	N	---------	Lived
17-M	21	--	30	N	N	---------	Lived

* Onset day of pathology.

† Pulmonary Involvement: S = severe; M = moderate; N = none.

¶ Degree of clinical sepsis: S = severe; M = moderate; N = none.

TABLE III

REDUCED IN VITRO GENERATION OF ANTIBODY FORMING CELLS BY SPLENOCYTES FROM THERMALLY INJURED MICE

Exp. #		Number of Direct AFC/10^6 Recovered Cells Generated by Cells Tested[a]				
		4 day[b] Post Burn	5 day Post Burn	6 day Post Burn	7 day Post Burn	8 day Post Burn
15	Thermally Injured	217±43.0	11±5.2	<1	<1	232±27.4
	Sham Injured	325±52.1	304±30.2	304± 66.9	313 ±45.5	401±29.9
22	Thermally Injured	33± 9.9	2±1.8	13± 1.4	58 ±10.8	120±23.9
	Sham Injured	285±34.1	421±18.3	223± 28.7	209 ±10.7	360±18.7
26	Thermally Injured	N.D.	1	1	N.D.	322±44.1
	Sham Injured		468±23.1	299±26.1		593±37.7
29	Thermally Injured	12± 4.6	N.D.	19± 6.3	99 ±22.0	267±63.9
	Sham Injured	327±54.8		363±60.1	203 ±25.6	385±35.1
		p[c]= .1	p=<.001	p=<.001	p=<.001	p=.025

[a] Avg. number of AFC generated in triplicate cultures assessed with Jerne slide assay (± standard error).

[b] Time after burn when animals were sacrificed and cultures. Two animals per group/day.

[c] Probability values for difference between thermally injured and sham injured calculated using the paired T test.

Table IV

Effect* of Added Mononuclear Cells† from Groups III§ Burn Patients on Normal High Responder MLR

Patient	Partial Reactions‖ ΔCPM**	Observed¶ ΔCPM	% Suppression
D	11,814	2,692	78
N	16,010	4,250	73
P	14,033	3,202	77
A	25,944	12,614	54
L	15,756	6,008	62
V	14,652	3,645	75
Q	19,562	7,230	63
E	14,355	4,480	69

* Effect of adding 10^5 burn cells or 10^5 mitomycin treated responder cells to "one way" MLR.

† Burn patients' mononuclear cells collected at 4-7 days post injury.

§ Group III consists of those burn patients who later developed acute sepsis (6/8 succumbed).

‖ R = norm responder; P = patient; S = norm stimulator. $\{R \times S_m + R \times P_m + P \times R_m + P \times S_m\}$.

¶ Avg ΔCPM triplicate cultures.

** $\{R \times S_m \times P\}$

Table V

Differences Between Observed Values* in Cultures with Added Cells and the Sums of the Partial Reactions†

Source of Added Mononuclear Cells			
Group I Burn Patients§	Group II Burn Patients	Group III Burn Patients	Normal Individuals
2,532 increase	2,380 increase	9,122 decrease	1,372 increase (+ 8%)¶
		9,748 decrease	3,373 increase (+ 16%)
1,302 decrease	1,814 decrease	9,875 decrease	
1,501 decrease	2,057 decrease	10,831 decrease	468 decrease (- 6%)
1,941 decrease	2,659 decrease	11,007 decrease	737 decrease (- 10%)
		11,766 decrease	810 decrease (- 11%)
		12,322 decrease	920 decrease (- 9%)
		15,330 decrease	

* ΔCPM ^{3}H-TdR of sum of the partial reactions minus R X S X Added cells (10^5).

† $\{R \times S_m + R \times P_m + P \times R_m + P \times S_m\}$ expressed as ΔCPM ^{3}H-TdR incorporation.

§ Cells collected at 4-7 days post injury for all burn patients.

¶ Percent of difference between observed CPM and Σpartial reactions.

Table VI

Correlation of Patient Suppressor Cell Sensitivity to Mitomycin* with Stimulator Activity of Patient Cells in MLR†

Day Post Injury	% Suppression P§	% Suppression P_m	CPM ^{3}H-TdR			
			R x P_m	R x S_m	P x R_m	P x S_m
5	70	32	11,247	10,879	4,822	654
7	73	75	4,250	11,322	242	196
5	57	18	2,988	11,292	3,888	3,732
8	62	58	2,933	9,632	1,532	286
12	26	0	3,558	7,182	4,123	2,807
5	74	42	13,490	10,345	1,775	955
9	66	56	2,624	16,376	1,056	648
2	11	2	17,773	25,254	18,335	1,900
8	79	18	5,920	11,287	2,764	623

* Cells from Group III patients incubated with 0.25 ug Mitomycin C before addition to cultures containing Normal "one-way" MLR combinations.

† Mitomycin treated patient cells stimulating normal responder cells.

§ Decrease in ΔCPM caused by addition of patient cells without Mitomycin treatment (P) or with treatment (P_m).

Table VII

Defective Activity of Ø After Thermal Injury

	$\bar{X}$ AFC/10^6 recovered cells	
	Exp 1	Exp 2
Burn Ø + Normal T-B	189	173
Sham Ø + Norm T-B	2030	1523
Norm Unseparated (Ø-T-B)	1229	1403
Norm T-B	1	0
Burn Ø Alone	10	55
Sham Ø Alone	60	90

Table VIII

Effect of Macrophage (Ø) Depletion on the

PHA Response Following Thermal Injury

	PHA Response (CPM ^{3}H-TdR)	
Days Post Injury	Undepleted	Ø Depleted
1	11,249	89,657
6	186	78,239
8	26,045	87,390
13	21,473	87,659
20	26,115	90,507
33	23,790	100,344

SPECIFIC AFC GENERATED FROM MIXTURES
OF AUTOLOGOUS BRONCHIAL WASH AND P.B. CELLS

ANTIBODY FORMING CELLS/10^6 RECOVERED CELLS				
UNFILTERED P.B.	FILTERED P.B.	UNFILTERED P.B. + Bronchial Wash*	FILTERED P.B. + Bronchial Wash*	
			10^5	5×10^5
0	0	0	31	---
0	0	0	20	92
0	0	0	18	65
10	0	21	60	227
20	9	15	---	208
17	0	25	---	239
0	0	0	20	89
10	0	12	---	163

*AFC of B.W. alone < 10.

Table IX

Table X

PARAMETERS OF IN VITRO GENERATION OF AFC FROM PB MONONUCLEAR CELLS IN CULTURE CONTAINING INDOMETHACIN

	$\bar{X}$ Specific AFC/10^6 Recovered Cells*							
	Fetal Calf Sera¶				AB Sera†			
	PB + Indomethacin**			PB Alone	PB + Indomethacin			PB Alone
	10^{-6}	10^{-7}	10^{-8}		10^{-6}	10^{-7}	10^{-8}	
ξZ	0	0	0	0	459	321	102	5
φS	6	17	27	0	95	261	161	0
φC	125	516	175	4	63	74	67	0
ξS	63	139	185	0	74	201	317	0
ζL	55	70	89	8	79	102	110	0
ΣP	102	133	65	0	93	307	188	0
ψM	90	108	79	0	441	232	170	8
ξS	83	201	122	0	58	101	82	13
φS	158	99	107	22	37	53	99	0
ψG	0	-	107	0	1625	2184	2742	84

* $\bar{X}$ AFC of duplicate cultures assayed in quadruplicate. Two x 10^6 cells cultures per well - 30 to 50% recovery.

¶ RPMI media contained 8% FCS.

RPMI media contained 8% SRBC absorbed pooled human AB sera.

** Final indomethacin concentration in culture (10^{-6} - 10^{-8} M).

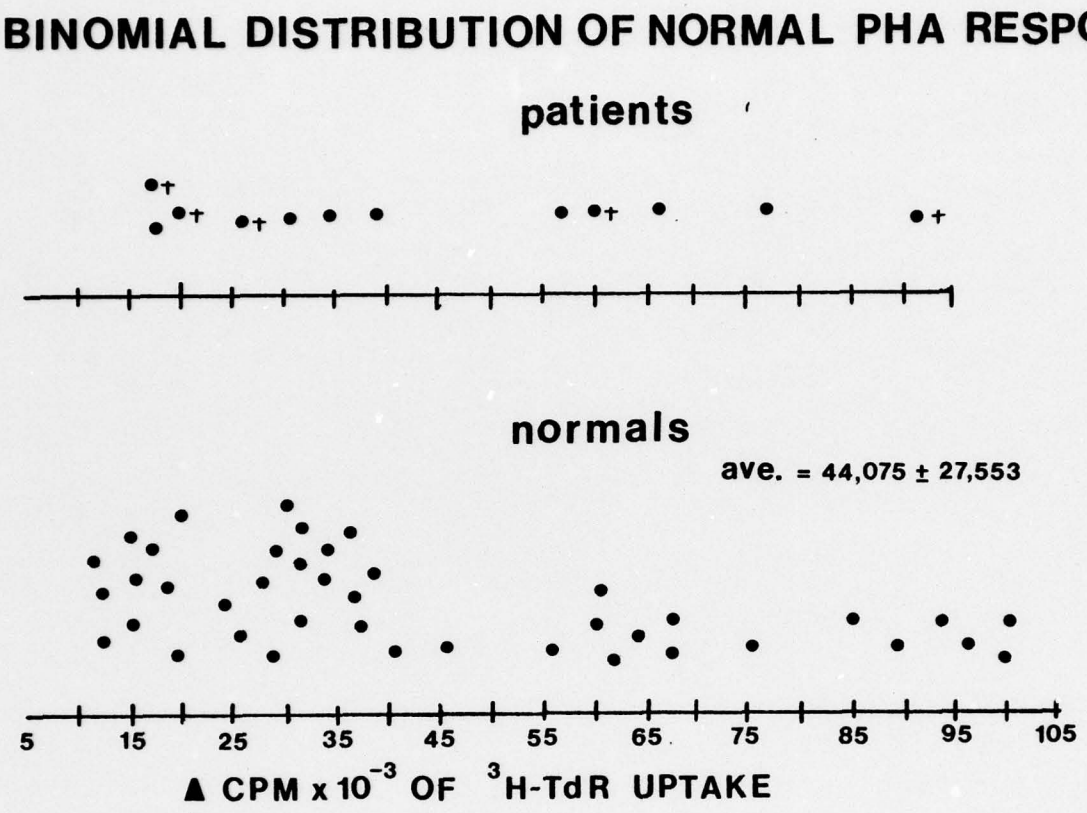
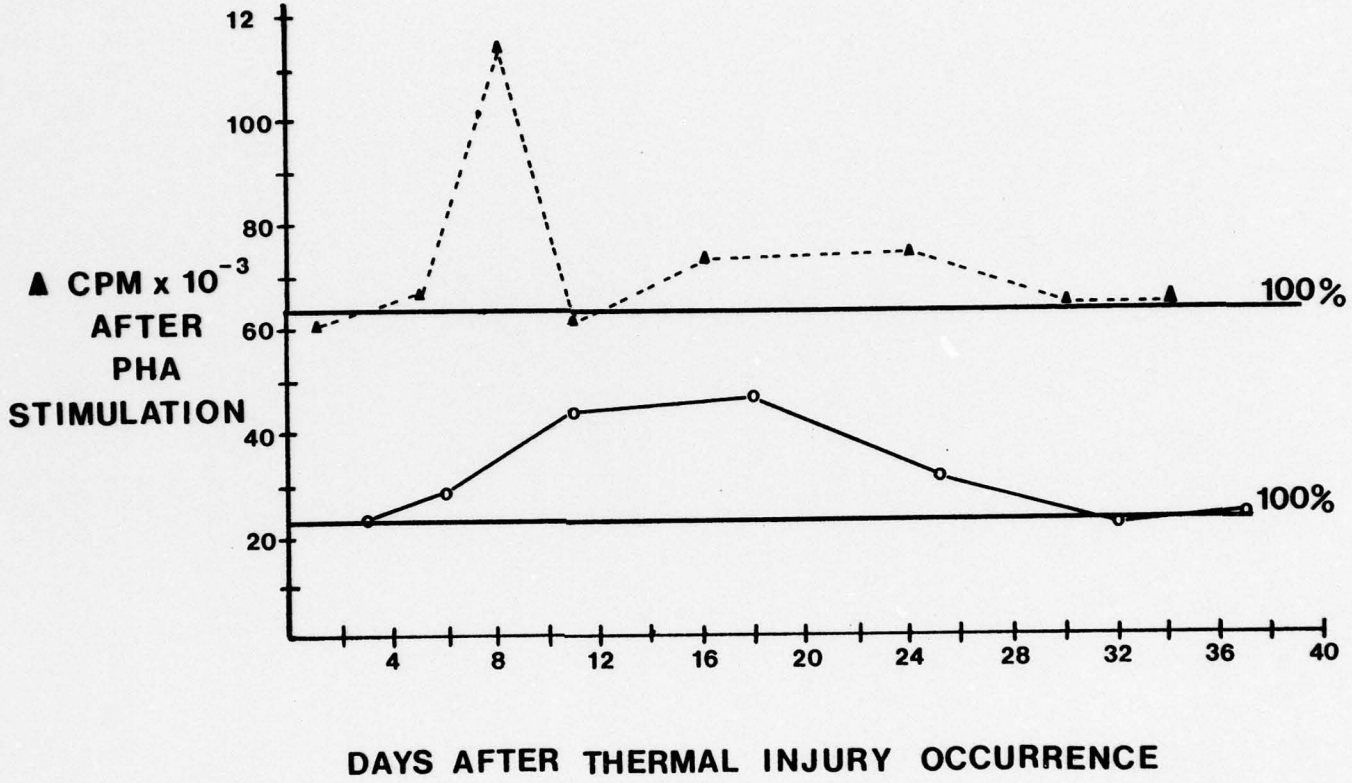

NON-BINOMIAL DISTRIBUTION OF NORMAL PHA RESPONSES
patients
normals
ave. = 44,075 ± 27,553
5
15
25
35
45
55
65
75
85
95
105
▲ CPM x 10⁻³ OF ³H-TdR UPTAKE

Figure 1

Figure 2

DIFFERENCES IN THE "NORMAL" PHA RESPONSE LEVELS OF THE MONONUCLEAR CELLS FROM TWO PATIENTS



PHA RESPONSES IN BURN PATIENTS WITH FATAL SEPSIS

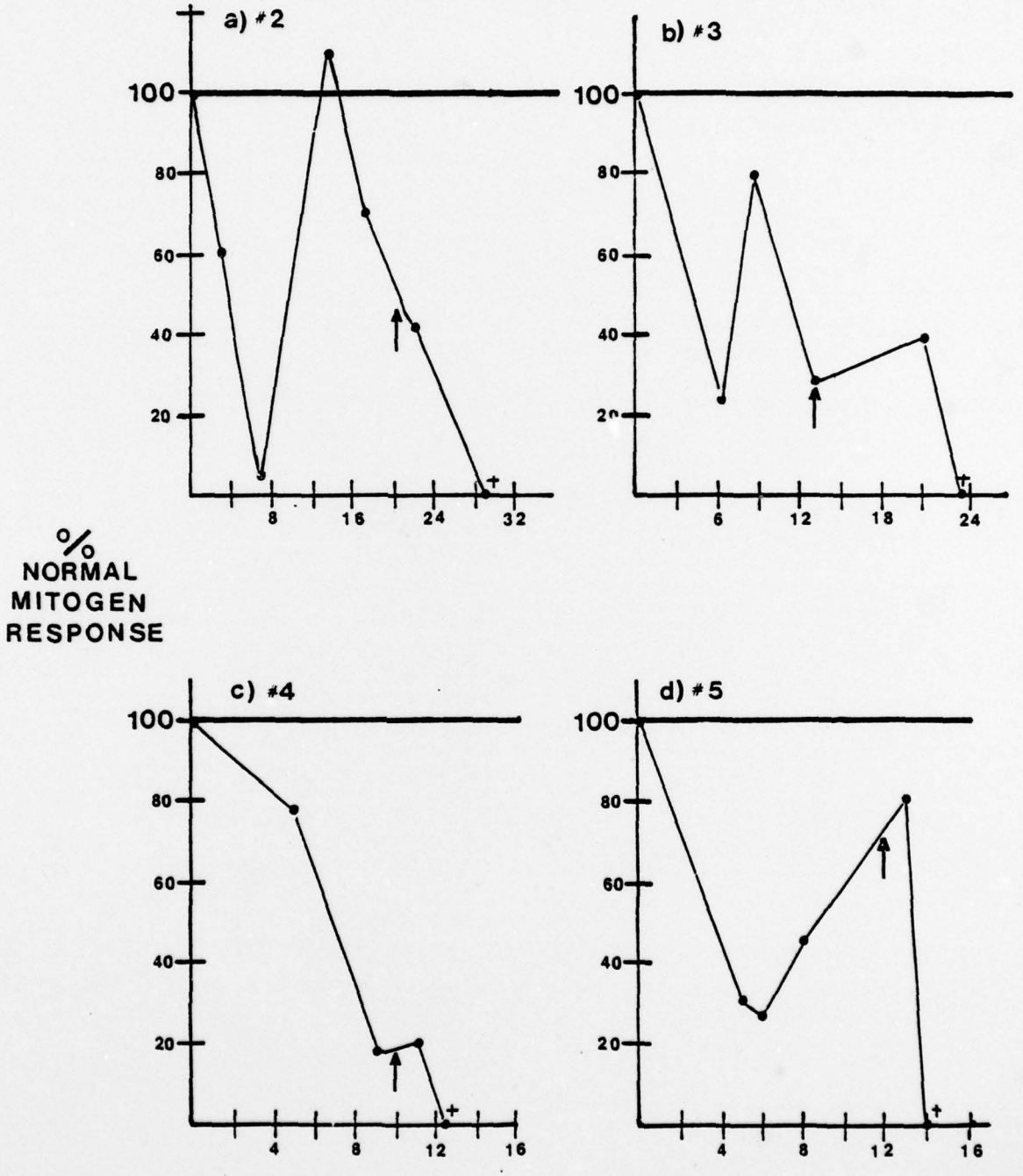


Figure 3

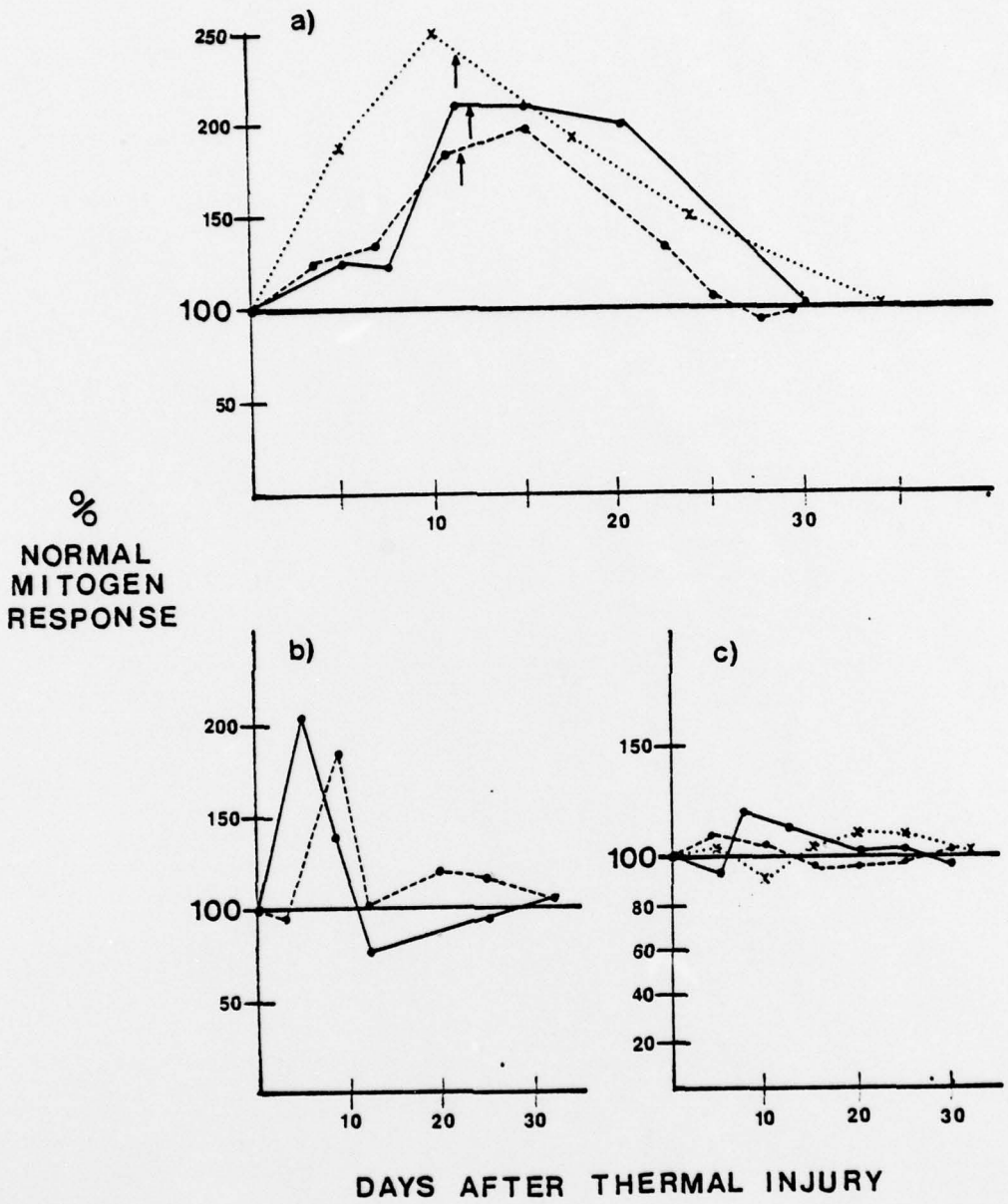

PHA RESPONSES AFTER THERMAL INJURY
a)
250
200
150
100
50
10
20
30
%
NORMAL
MITOGEN
RESPONSE
b)
200
150
100
50
10
20
30
c)
150
100
80
60
40
20
10
20
30
DAYS AFTER THERMAL INJURY

Figure 4

PHA RESPONSES IN BURN PATIENTS SURVIVING SEPSIS

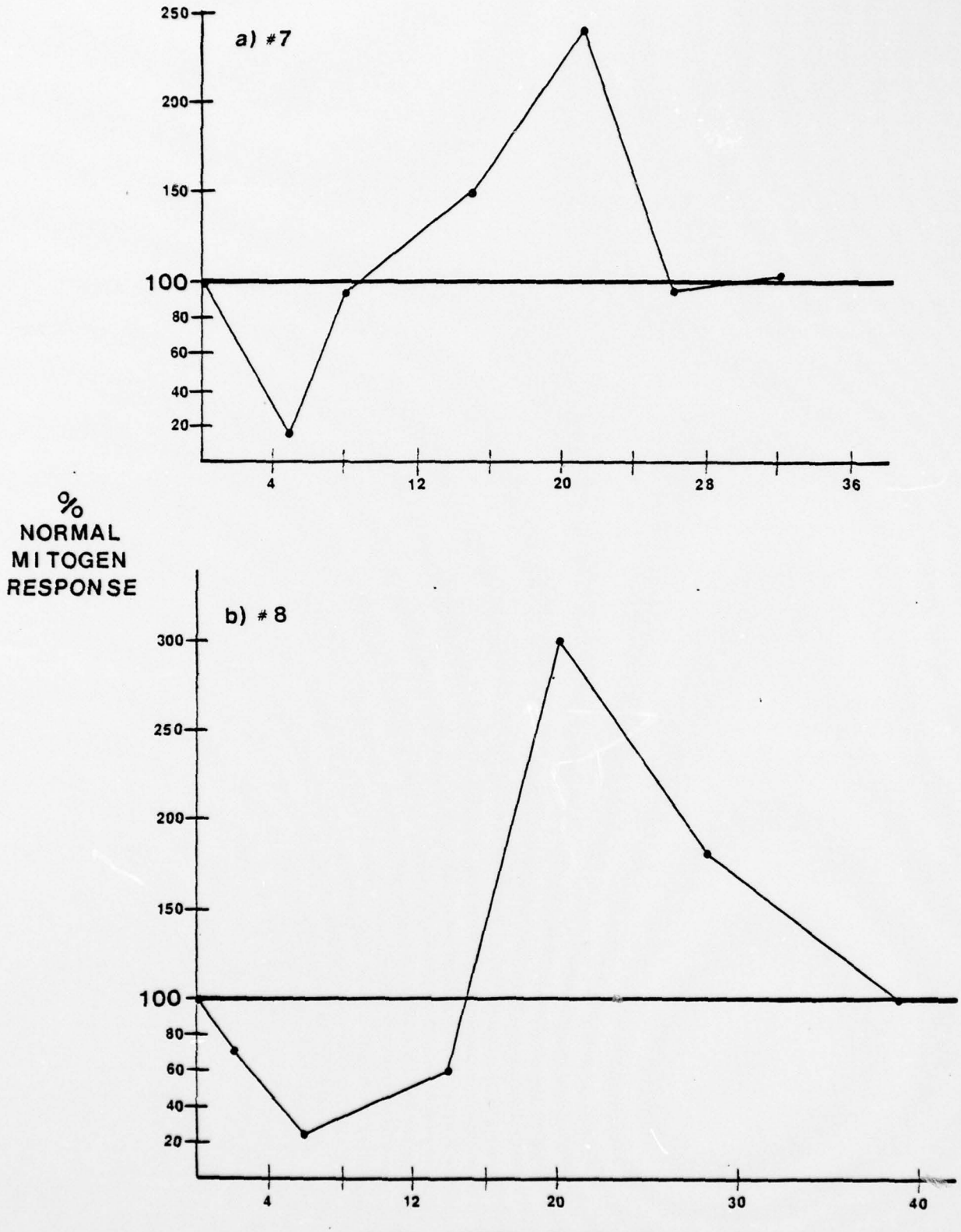


Figure 5

PHA RESPONSE PATTERNS AFTER THERMAL INJURY

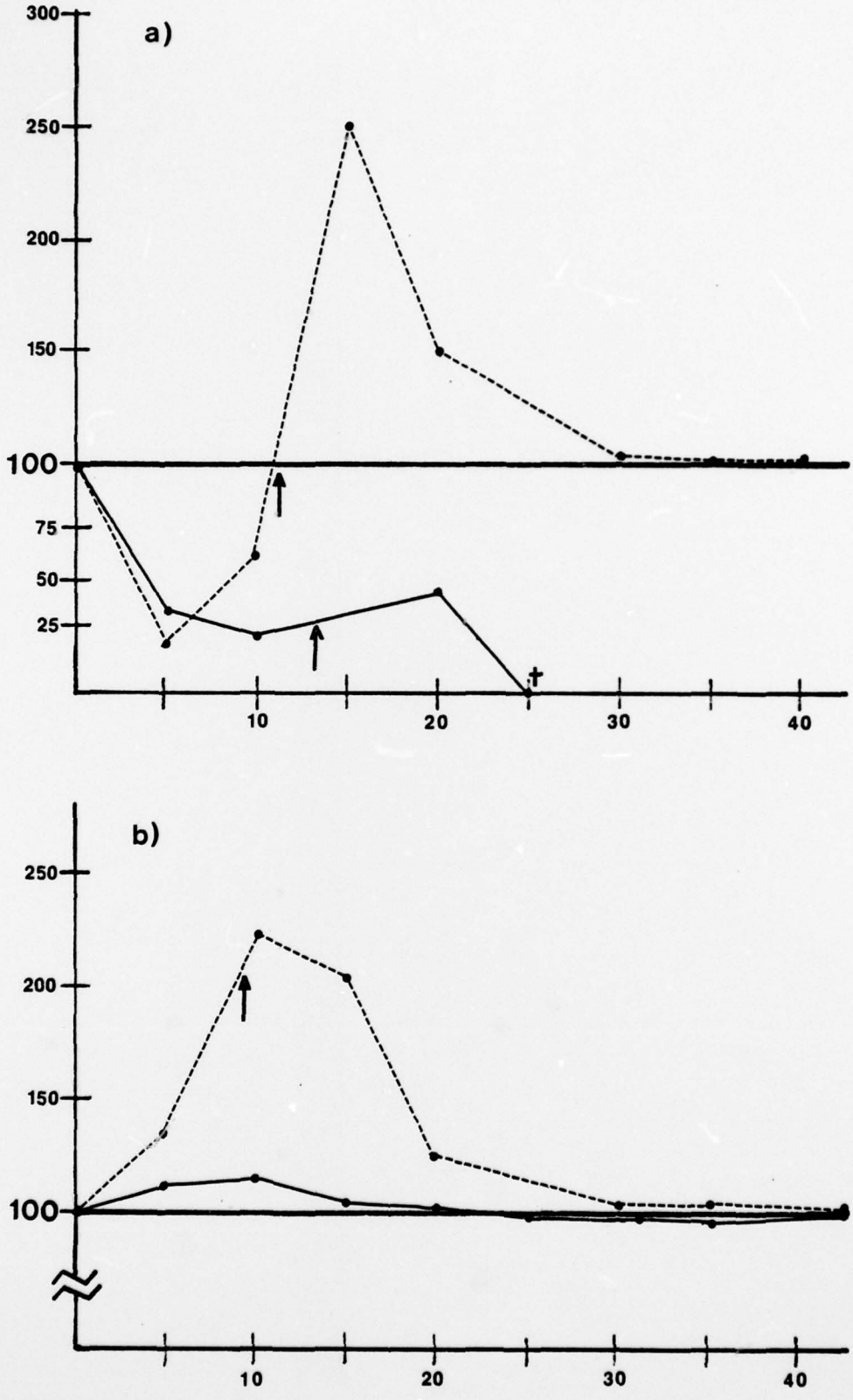


Figure 6

SUPPRESSION OF NORMAL MLR BY BURN PATIENT CELLS

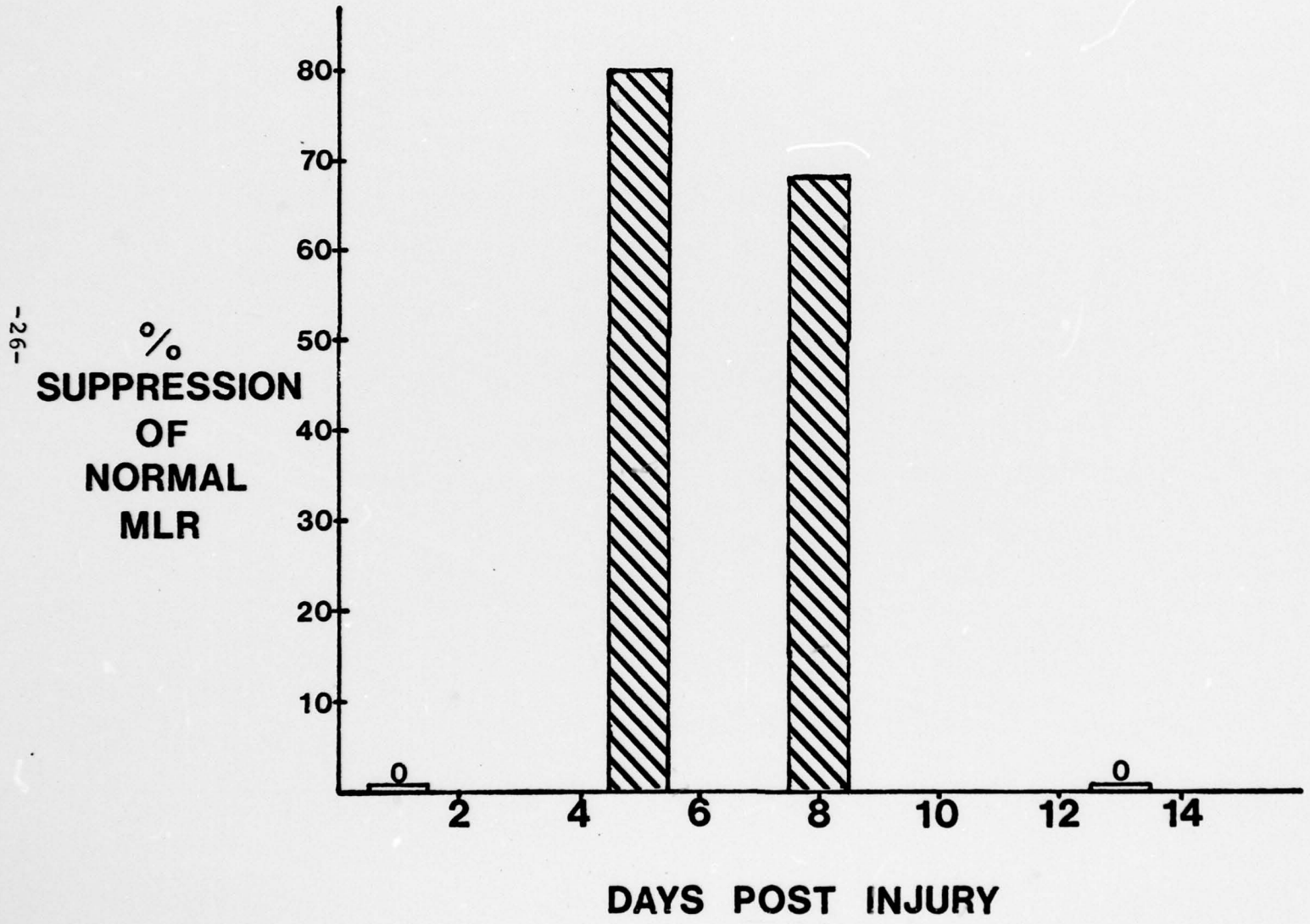


FIG. 7

Figure 8

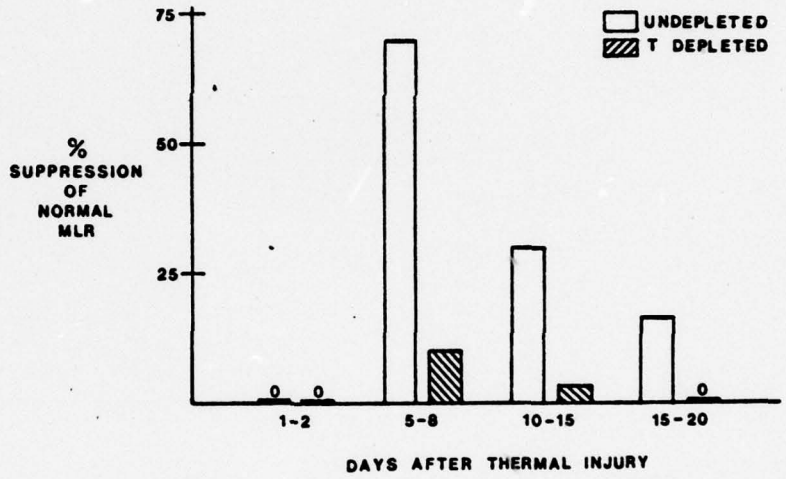
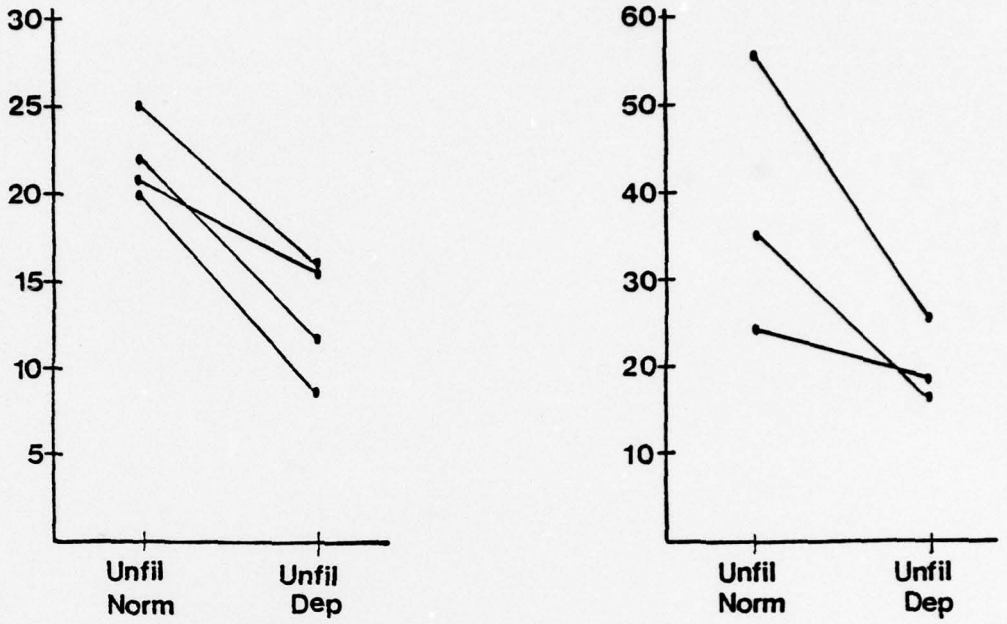


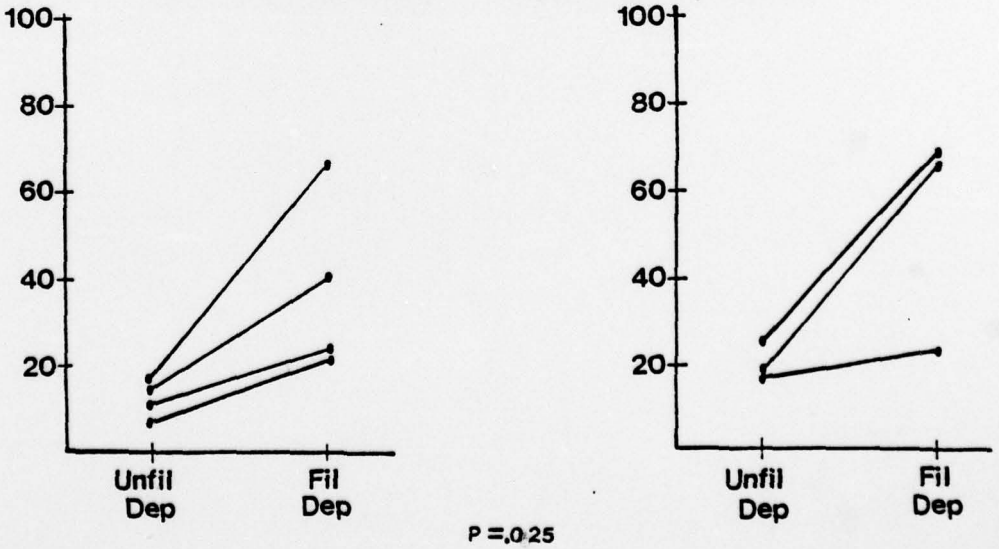
FIG. 2. Removal of suppressive activity from mononuclear cells of burn patients by T-cell depletion. Percentage suppression represents the percentage decrease in proliferation of the normal "one-way" MLR following addition of patient cells collected at various times after injury. The clear bars represent the suppressive activity of untreated patient cells, and the cross-hatched bars illustrate the decrease in suppressive activity when patient cells are depleted of T cells and then added to the normal MLR.

DEPRESSION OF SPLENECTOMY PATIENTS' PHA RESPONSE AT 10 TO 15 DAYS AFTER SURGERY



▲ CPM
^{3}H-TdR
INCORPORATED

REVERSAL OF HYPORESPONSIVENESS BY DEPLETION OF INHIBTORY MACROPHAGE



P =.025

Figure 9

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Recent Publications of Work Supported by this Contract:

1. Miller CL: 1978. Immunological assays as measurements of nutritional status - A review. J Parent Enter Nutr 2:554.

2. Miller CL, Baker C: 1979. Changes in lymphocyte activity after thermal injury: Role of suppressor cells. J Clin Invest (in press).

3. Miller CL, Claudy B: 1979. Suppressor T cells induced by thermal injury. Cellular Immunol (in press).

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