STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY SEVERE INJURY

MASSACHUSETTS UNIV MEDICAL SCHOOL

WORCESTER DEPT OF SURGERY

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STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY SEVERE INJURY

ANNUAL REPORT

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Studies of altered response to infection induced by severe injury

Carol L. Miller, Ph.D.

This year has greatly expanded our understanding of the development of immuno-incompetence in trauma patients and suggests the usefulness of clinical trials with PGE2 inhibitors. Our preliminary data on the relationship between an early (3 days post injury) shift in identifiable MØ subset ratio and the development of immuno-incompetence have importance to the rapid detection of patient immuno-incompetence. If we can firmly establish that measurement of the ratio alone is sufficient to predict increased risk of sepsis, then this assessment has potential use in military care of the wounded. A simple assay for determining the ratio of $\text{Fe}^+\text{MØ}$ to $\text{Fe}^-\text{MØ}$ is easily developed. Such an assay would only require drawing a very small blood sample. The peripheral blood could be rapidly segregated on lymphoprep or other commercial preparations, then $\text{MØ}$ isolated by adherence to prepared Akerman Douglas plates for 2 hrs., then labeled with anti $\text{Fe}$ fluorescent labeled antibody. Counting the number of fluorescently labeled $\text{MØ}$ in a 100 $\text{MØ}$ total could then be performed in a very short time resulting in a relevant clinical monitoring tool.
SUMMARY

This year has greatly expanded our understanding of the development of immuno-incompetence in trauma patients and suggests the usefulness of clinical trials with PGE inhibitors. Our preliminary data on the relationship between an early (3 days post injury) shift in identifiable MØ subset ratio and the development of immuno-incompetence have importance to the rapid detection of patient immuno-incompetence. If we can firmly establish that measurement of the ratio alone is sufficient to predict increased risk of sepsis, then this assessment has potential use in military care of the wounded. A simple assay for determining the ratio of Fc

MØ to Fc

MØ is easily developed. Such an assay would only require drawing a very small blood sample. The peripheral blood could be rapidly segregated on Lymphoprep or other commercial preparations, then MØ isolated by adherence to prepared Akerman Douglas plates for 2 hrs., then labeled with anti Fc fluorescent labeled antibody. Counting the number of florescently labeled MØ in a 100 MØ total could then be performed in a very short time resulting in a relevant clinical monitoring tool.
In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).

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

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

The goals of his research are (1) to delineate the role of suppressor T cells and inhibitory monocytes (MØ) in mediating the immunosuppression that follows severe injury, (2) to develop and improve assays for assessment of macrophage function, (3) to investigate the relationship between immunosuppression and post-trauma alterations in MØ subsets, and to investigate prophylactic modalities for reversing immunosuppression.

In pursuing goal one of this contract period, we have demonstrated that trauma induced suppressor T cells (Tₜ) can depress monocyte function and that at least some of the Tₜ mediate immunosuppression by stimulating monocytes to produce elevated levels of prostaglandin E₂ (PGE₂). In pursuing goal two, we have concentrated on trying to associate a change in MØ function with a change in MØ phenotype. We have shown that elevation of MØ PGE₂ production appears to correlate with the shift toward a MØ subset that can be identified by its expression of a high affinity receptor for the crystaline fragment of Immunoglobulin G (i.e., Fc⁺). If it is possible to develop a phenotype profile of a defective MØ, then it would no longer be necessary to do functional assays on patients' blood. Instead, a small quantity of blood could be drawn, the MØ rapidly isolated (2 hours) and stained with fluorescent antibodies which were directed against the surface markers known to identify a particular subset. The increased appearance of this subset could then be enumerated by a simple counting technique allowing quick detection of the onset of immunoincompetence at far forward locations. It is for this reason that we have pursued goal three. We have demonstrated that there is an increase in the numbers of the Fc⁺ MØ subset and that this increase correlates both to elevation of PGE₂ production and poor clinical outcome. Finally, in testing of possible new prophylactic
therapies, we have been initially stymied by the failure to have our burned guinea pig model approved by the Animal Research Committee. However, after making several changes in the protocol, we feel that approval should be granted in the next contract year. We have, however, examined the effect of a monocyte stimulator (muramyl dipeptide) on patient MØ function in vitro. Our results suggest that this agent increases MØ PGE₂ production and is contra-indicated in patients who have already undergone a shift in their MØ subset ratios toward a larger proportion of PGE₂ producers. There had been some reports in the literature suggesting the use of MDP as an immunopotentiator.

In summary, this has been a highly productive year in which we have made progress on many of the goals of the five year contract. Our most significant finding is probably the relationship between elevation of immunocompromised patient MØ PGE₂ production and a shift in the patient MØ subset ratios.

**Methods**

The Plasminogen Activator (PA) assay is performed as previously described (1). The procoagulant assay and the lysozme assay are also performed as previously described (1-2). The assay for suppression of MØ PA activity is performed by isolating the MØ as described (1), then recombining either $2 \times 10^6$ normal or $2 \times 10^6$ patient MØ with equal numbers ($2 \times 10^6$) of the patients E-rossetted T cells. The mixed populations are co-cultured for 48 hours in Iscove's supplemented with 8% FCS and media supplements as described (1). In most assays, the normal and patient MØ are collected at day 1-5 post injury and cultured in supplemented Iscove's for 3-4 days. The patient T cells collected at 5-9 days post injury are co-cultured with the normal or patient MØ collected 3-4 days earlier.

MØ PGE₂ is measured with a standard RIA as previously described (3). Leukocyte pyrogen is assessed in a modification of the Bodell assay (4). In this modification, 250 gm Balb/c mice are pre-warmed to 37⁰, and their baseline temperatures determined over a 20-minute period. The mice then receive a 0.20 ml IV injection of supernates collected from the patients or normal MØ cultures.
The elevation of the mice temperatures is recorded over a 50-minute period with a Yellow Springs Thermoprobe and chart recorder. A media control is also always included.

The sensitivity of MØ to Muramyl Dipeptide is assessed by culturing isolated MØ with 20 µg/ml of MDP for 48 hours in a protocol identical to that we have previously used in experiments with peptidoglycan (5). The PA, PGE₂, Lysozyme, and procoagulant activities of the patient and normal MØ are compared in the presence and absence of MDP. The measurement of the MØ LP levels in the MDP stimulated MØ culture supernates has presented special problems. The correct control is media + MDP. However, both this control and the normal MØ supernates contain residual MDP which in and of itself causes elevation of the mice temperatures. Although the kinetics of this MDP induced pyrogen activity are different than that of LP (LP peak 20 min., MDP peak > 70 min.), the overlap complicated data interpretation.

As previously reported in our quarterly, we now use a protocol whereby the mice are made MDP tolerant by 3 days of injection of increasing MDP doses. When the animals are no longer responsive to MDP, they are used to assess the LP activity of the MDP containing MØ supernates. Using this technique, we have derived significant information on MØ LP stimulation by MDP.

Three methods are used to generate subsets of MØ. All three are rosetting based protocols. In order to isolate MØ positive for the high affinity Fc receptor for IgG, we rosette with anti-Rh antibody coated human erythrocytes using the method of Zembala et al (6). The method for rosetting a population of DQ⁺ MØ involves CrCl linking of anti DQ monoclonal antibody to Ox red cells following the method of Mills et al (7). The third method of generating MØ subsets involves treatment of MØ with anti-CR (C3b, C4b receptors) antibody and rosetting the CR negative MØ using anti sheep red blood cell coated erythrocytes with complement compounds 1, 4, 2, 3 on their surface (8).
Results and Discussion

In the period covered by the first year of contract No. DAMD17-86-C-6091, several goals have been attained. Thirty-one patients have been nominated this year. The patients included 19 burn and 11 trauma patients.

Of these patients, twelve were studied the first four months, eight the next three months, seven the third three months, and four the last two months of the year. Of these patients, three burn patients and two trauma patients have succumbed to fatal sepsis. The small number of trauma patients studied is a result of our consistent exclusion of head trauma. Most of the patients are admitted to the trauma center with significant head trauma. Consequently, in the coming year, patients with significant trauma and moderate head trauma will be studied as separate subsets of our trauma patient group.

As we have previously shown, the trauma patients who experienced immunodepression had reduced monocyte (MØ) plasminogen activator (PA) activity with simultaneously elevated MØ PGE$_2$ and leukocyte pyrogen (LP). As illustrated in Table 1, there is a consistent correlation between (1) MØ PA depression and (2) depression of phytohemagglutinin (PHA) induced T cell melanogens concomitant with (3) PGE$_2$ elevation of MØ prostaglandin E$_2$ (PGE$_2$) levels and (4) poor prognosis. As can be seen, there is little change in MØ PGE$_2$ in either trauma or burn patients who experience no immunodepression or septic complications. The data showing PGE$_2$ to be correlated to immunodepression has lead us to re-examine the excessive T lymphocytic suppressor cell activity in immunocompromised trauma patients. We examined the affect of patients’ isolated T cells on monocyte responses. Both the patients own and normal monocytes were assessed. The patients’ MØ are isolated at day one post injury, then cultured for three days in Iscove’s medium. Normal controls are similarly cultured for three days. On day 4-5 post injury, the patients’ T cells are isolated and added to both patient and normal cultures. After a further two-day incubation, the response of the normal and patients’ MØ without added T cells are compared to the patient and normal cultures which have been incubated two days with
patients' T cells. The patients' T cells collected at 3-7 days post injury consistently suppressed the patients' own MØ plasminogen activator responses, as can be seen in Table 2, Fig. 1. Patients' T cells collected at greater than day 6 post injury consistently suppressed normal MØ PA responses. However, the suppressive activity for normal MØ PA production of patient T cells collected earlier in the post trauma course was variable. These data suggest some genetic restriction on the action of Tₕ collected at an earlier post injury period. The depression of MØ PA by patient T cells does not appear to result from protein synthesis depression or from cytotoxicity. As also illustrated in Table 2, Fig. 1, and Table 3, the PGE₂ production and leukocyte pyrogen (LP) activity of these immunosuppressed patient MØ are augmented simultaneously to depression of MØ PA activity.

One possible mechanism by which patient T cells could non-specifically suppress normal MØ function is by increasing the MØ PGE₂ levels. In previous years, we have reported an overall increase in PGE₂ production in patients who experienced immunoincompetence. In our recent experiments, we have examined the ability of patients' Tₕ to increase PGE₂ production. As illustrated in Table 2, Fig. 1, the patient T cells isolated at day 1 post injury are not inhibitory to the PA response nor do they exhibit increased PGE₂ production. However, T cells from the same patient collected at 6-13 days post injury suppressed normal MØ PA, increased MØ PGE₂ levels, and increased normal MØ leukocyte pyrogen (LP) activity. These data imply that increase in MØ PGE₂ production may significantly contribute to immunosuppression of trauma patients. In addition, at least one of the mechanisms for post trauma increases in MØ PGE₂ is the action of Tₕ on MØ. Increased PGE₂ levels will depress MØ PA production and MØ antigen presenting capacity thereby increasing Tₕ generation.

As already illustrated, patients' MØ populations exhibit much higher PGE₂ levels than can be induced in normal MØ populations. This apparent increased sensitivity to PGE₂ induction would result if trauma induced (via complement split products, fibrin degradation products or leukocyte mediators) a shift in
the proportion or ratio of the MØ subset that produces PGE$_2$. Previous investigators have indicated that the MØ population is functionally heterogeneous and that this functional heterogeneity is associated with certain MØ subsets. Alternately, trauma could increase the ability of each individual MØ to produce PGE$_2$.

We have examined the stimulation capacity of Muramyl Dipeptide (MDP) on patient and normal MØ to detect any post-trauma change in MØ responsiveness to bacterial stimuli. MDP is a potent PGE$_2$ secretagogue and we, and others, have found that it also depresses MØ PA activity. As can be seen in Table 4, 20 $\mu$g/ml of MDP stimulates maximal PGE$_2$ activity in the range of 50,000 pg for both normal and patient day 1 MØ. Increasing the dose of MDP to 30 $\mu$g/ml failed to increase the level of PGE$_2$ above this range. In striking contrast, the patient MØ collected at >Day 6 post were continually producing high levels of PGE$_2$, and when stimulated with MDP, increased these levels by 2 to 3 fold. The MØ PA responses of these patients already appeared to be maximally suppressed and did not additionally decrease their PA and responses upon MDP stimulation. These data indicate either a post-trauma increase in a PGE$_2$ producing subset or an increased capacity for PGE$_2$ production in all MØ.

We examined the subset ratios of patients MØ populations using the MØ subset with a high affinity receptor for IgG2 (Fc$^+$). This Fc$^+$ subset has been previously suggested to contain the inhibitory MØ population (6). We, therefore, examined the PGE$_2$ production and Fc$^+$ subset proportions in immunosuppressed trauma patients. As can be seen in Figures 2 and 3, immunodepressed patients had a dramatic increase in the proportion of the Fc$^+$ MØ which coincided with an increase in their PGE$_2$ activity. The Fc$^+$ MØ were the primary, if not the sole, MØ producers of PGE$_2$. The normal MØ and immunocompetent patient MØ populations contained approximately 40% high affinity Fc$^+$ MØ and 60% Fc$^-$ MØ after isolation on Ackerman Douglas Flasks. We have now examined a number of patients over time post injury. Those patients who survived multiple septic episodes and regained immunocompetence showed a return
to normal Fc+/Fc- MØ ratio levels (Table 5). Trauma patients with normal PA responses had normal Fc+/Fc- MØ ratios. Interestingly, patients with depressed MØ PA had normal PA responses when only their isolated Fc- monocytes were assessed in the absence of the Fc+ subset. Similarly, the patients late Tₕ were unable to suppress either the patients Fc- or normal Fc- MØ PA responses. Only Fc+ responses were suppressed (Table 6). These data support the idea that some of the Tₕ suppression mechanism may be partially a result of increasing MØ PGE₂ activity.

We have also examined the leukocyte pyrogen (LP) responses of the Fc+ and Fc- subsets and found that LP activity is confined primarily to the Fc+ MØ subset (Table 7). Addition of immunosuppressed patient Tₕ to normal or day 1 post injury patient MØ was found to increase the MØ LP activity as well as depress the MØ PA activity.

In summary, these data further support our hypothesis that a shift in the trauma patient Fc+ to Fc- MØ ratio increases overall patient PGE₂ production. Furthermore, excessive Tₕ generation may also contribute to elevating MØ PGE₂ levels and development of immunoincompetence. Several questions on the mechanism by which trauma could mediate immunosuppression are currently being explored. It is possible that immunosuppressive patient Fc- MØ are altered so that they can respond to MDP with PGE₂ production. We have already shown that normal Fc- monocytes can not be stimulated by MDP to produce PGE₂ or LP. However, we do not as yet have data indicating that patient Fc- MØ do not respond to MDP stimulation by increasing their PGE₂ or LP output. It also must be noted that the MØ subset we are calling Fc- is not, in fact, devoid of Fc receptors. The anti RH antibody consists primarily of IgG₂. Human monocytes have two receptors for IgG, a "high affinity" receptor with a preference for IgG₂, and a "low affinity" receptor with a preference for IgG₁. When we utilized a specific anti low affinity Fc receptor antibody, we found that most (if not all) MØ have the low affinity Fc receptor, but only a subset express the high affinity. Consequently, the Fc+ population we are describing in actuality
is the high affinity positive population. Nevertheless, our data show that this high affinity positive MØ subset is increased at the expense of the low affinity (Fc\(^-\)) subset in immunocompromised trauma patients. This high affinity Fc\(^+\) MØ subset is enriched for both LP and PGE\(_2\) producers but not for PA producers. The result of this shift in MØ subset Fc\(^+\)/Fc\(^-\) ratio is that patients who develop immunoincompetence show increased PGE\(_2\) production and respond to bacterial stimuli (MDP) with a greater production of PGE\(_2\) than normal individuals’ MØ. Furthermore, T cells from immunoincompetent patients can depress facilitory MØ functions (i.e. PA) and increase MØ PGE\(_2\) levels thereby further perpetuating the immunosuppressive cycle.

This year has greatly expanded our understanding of the development of immuno-incompetence in trauma patients and suggests the usefulness of clinical trials with PGE\(_2\) inhibitors. In addition, these preliminary data are on the relationship between an early (3 days post injury) shift in identifiable MØ subset ratio and the development of immunoincompetence have importance to the rapid detection of patient immunoincompetence. If we can firmly establish that measurement of the ratio alone is sufficient to predict increased risk of sepsis, then this assessment has potential for army use. A simple assay for determining the ratio of Fc\(^+\) MØ to Fc\(^-\) MØ is easily developed. Such an assay would only require drawing a very small blood sample. The peripheral blood could be rapidly segregated on Lymphoprep or other commercial preparations, then MØ isolated by adherence to prepared Akerman Douglas plates for 2 hrs., then labeled with anti Fc fluorescent labeled antibody. Counting the number of fluorescently labeled MØ in a 100 MØ total could then be performed in a very short time resulting in a relevant clinical monitoring tool.
References


8. Dobson NJ, Lambris JD, Ross GD: Characteristics of isolated erythrocyte complement receptor type one (CR\textsubscript{1}, C4b-C3b receptor) and CR\textsubscript{1}-specific antibodies. J. Immunol. 1981. 125, 693-698.
TABLE 1

Correlation MØ and T Cell Depression and Clinical Outcome

<table>
<thead>
<tr>
<th>Pt</th>
<th>Injury</th>
<th>ΔPHAa</th>
<th>PA(0-13)b</th>
<th>ΔPGE2 x 10^{-3} pgc</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ja</td>
<td>Trauma</td>
<td>55</td>
<td>41.8 → 14.1</td>
<td>1.9 → 15.4</td>
<td>Multiple septic episodes</td>
</tr>
<tr>
<td>Sw</td>
<td>Trauma</td>
<td>-90</td>
<td>26.8 → 8.6</td>
<td>1.5 → 12.8</td>
<td>Fatal sepsis</td>
</tr>
<tr>
<td>Ch</td>
<td>Trauma</td>
<td>-20</td>
<td>34.1 → 22.0</td>
<td>8.4 → 6.8</td>
<td>No complications</td>
</tr>
<tr>
<td>Br</td>
<td>Trauma</td>
<td>-15</td>
<td>18.0 → 22.2</td>
<td>4.3 → 1.6</td>
<td>No complications</td>
</tr>
<tr>
<td>Pr</td>
<td>Trauma</td>
<td>-20</td>
<td>32.9 → 24.4</td>
<td>12.3 → 12.3</td>
<td>No complications</td>
</tr>
<tr>
<td>Mu</td>
<td>Burn</td>
<td>-87</td>
<td>48.8 → 7.2</td>
<td>1.6 → 23.6</td>
<td>Fatal sepsis</td>
</tr>
<tr>
<td>Ke</td>
<td>Burn</td>
<td>-59</td>
<td>32.1 → 12.9</td>
<td>2.7 → 11.3</td>
<td>Multiple septic episodes</td>
</tr>
<tr>
<td>Ar</td>
<td>Burn</td>
<td>-82</td>
<td>45.4 → 19.1</td>
<td>5.9 → 14.2</td>
<td>Multiple septic episodes</td>
</tr>
<tr>
<td>Be</td>
<td>Burn</td>
<td>-79</td>
<td>41.6 → 15.0</td>
<td>5.3 → 12.5</td>
<td>Fatal sepsis</td>
</tr>
<tr>
<td>Ho</td>
<td>Burn</td>
<td>-80</td>
<td>39.2 → 8.2</td>
<td>3.3 → 13.9</td>
<td>Multiple septic episodes</td>
</tr>
<tr>
<td>Cr</td>
<td>Burn</td>
<td>-78</td>
<td>31.8 → 14.7</td>
<td>2.6 → 11.1</td>
<td>Fatal sepsis</td>
</tr>
<tr>
<td>Gh</td>
<td>Burn</td>
<td>+30</td>
<td>44.2 → 39.9</td>
<td>5.8 → 5.0</td>
<td>No complications</td>
</tr>
<tr>
<td>Jo</td>
<td>Burn</td>
<td>+100</td>
<td>26.1 → 21.5</td>
<td>7.0 → 8.8</td>
<td>One infectious episode</td>
</tr>
<tr>
<td>Ro</td>
<td>Burn</td>
<td>+12</td>
<td>32.4 → 27.6</td>
<td>2.8 → 2.6</td>
<td>No complications</td>
</tr>
<tr>
<td>Nu</td>
<td>Burn</td>
<td>-20</td>
<td>25.0 → 18.9</td>
<td>6.7 → 9.9</td>
<td>No complications</td>
</tr>
</tbody>
</table>

a. Maximum change at 4-13 days post injury in mitogen induced proliferation of 2 x 10^6 peripheral blood mononuclear cells to 0.2 mg PHA

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

c. Maximum prostaglandin E, production in first 13 post injury days measured as picograms per 10^6 recovered MØ (x10^{-3})
TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>PGE₂</th>
<th>pg/10⁶ MØ</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norm MØ + D1 PT T-Cells c</td>
<td>9,025</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>Norm MØ</td>
<td>13,544</td>
<td>27.1</td>
<td></td>
</tr>
<tr>
<td>Norm MØ + D&gt;6 T-Cell</td>
<td>45,451</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>Norm MØ</td>
<td>22,610</td>
<td>34.5</td>
<td></td>
</tr>
</tbody>
</table>

a. T lymphocytes were isolated from patient mononuclear preparations by E rosetting of MØ depleted populations.

b. PGE₂ in picograms per 10⁶ recovered MØ.

c. 2 x 10⁶ MØ were cocultured 2 days with 2 x 10⁶ patient (pt) T lymphocytes collected at day 1 post-injury (D1).
### Table 3

**EFFECT OF PATIENT T CELLS ON MØ FUNCTION**

<table>
<thead>
<tr>
<th>CELLS</th>
<th>LP Δtemp&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
<th>PA%&lt;sup&gt;b&lt;/sup&gt;</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Exp 112</td>
<td>Exp 130</td>
<td></td>
<td>Exp 112</td>
<td>Exp 130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norm MØ</td>
<td>.3</td>
<td>.15</td>
<td></td>
<td>59.5</td>
<td>52.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norm MØ + Pt T Cell&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.35</td>
<td>.20</td>
<td></td>
<td>37.7</td>
<td>22.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt MØ</td>
<td>.35</td>
<td>.65</td>
<td></td>
<td>21.5</td>
<td>49.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt MØ + Pt T Cell&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.75</td>
<td>.85</td>
<td></td>
<td>10.5</td>
<td>13.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **a.** Leukocyte pyrogen (LP) was assessed as change in temperature (Δtemp) of mice injected with 0.3 ml MØ supernate
- **b.** MØ production of plasminogen activator (PA) is measured in percent specific fibrinolysis
- **c.** $2 \times 10^6$ normal individual's MØ were cocultured for 2 days with $2 \times 10^6$ patient (Pt) T lymphocytes
- **d.** $2 \times 10^6$ patient MØ isolated at day 1-3 post-injury and maintained in culture were then cocultured for 2 days with the Pt T cells collected 4-6 days post-injury
**Table 4**

**EFFECT OF MDP\(^A\) ON NORMAL OR PATIENT M\(^b\) FUNCTION**

<table>
<thead>
<tr>
<th>Mo</th>
<th>PGE(_2)^b</th>
<th>PA(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norm</td>
<td>16,944 ± 6,717</td>
<td>31.4 ± 7.4</td>
</tr>
<tr>
<td>Norm + MDP</td>
<td>54,387 ± 23,030</td>
<td>15.2 ± 3.8</td>
</tr>
<tr>
<td>D1 Pt</td>
<td>19,759 ± 7,295</td>
<td>33.5 ± 12.1</td>
</tr>
<tr>
<td>D1 Pt + MDP</td>
<td>49,049 ± 22,529</td>
<td>13.8 ± 4.2</td>
</tr>
<tr>
<td>D&gt;6 Pt</td>
<td>37,940 ± 11,606</td>
<td>14.4 ± 4.1</td>
</tr>
<tr>
<td>D&gt;6 Pt + MDP</td>
<td>91,851 ± 20,951</td>
<td>14.0 ± 1.6</td>
</tr>
</tbody>
</table>

---

A. 2×10\(^6\) M\(^b\) CULTURED FOR 2 DAYS WITH 20\(\mu\)G/ML OF MURAMYL DIPEPTIDE (MDP)

B. PROSTAGLANDIN E\(_2\) PRODUCTION IN PICOGRAMS PER 10\(^6\) RECOVERED M\(^b\)

C. PLASMINOGEN ACTIVATOR ACTIVITY IN % SPECIFIC FIBRINOLYSIS.
TABLE 5

Change on MØ Fc⁺/Fc⁻ subset ratios over time post injury

<table>
<thead>
<tr>
<th></th>
<th>% Fc⁺/Fc⁻</th>
<th>Fc⁺</th>
<th>Fc⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>PtT day 15</td>
<td>75/25</td>
<td>38±6</td>
<td>62±6</td>
</tr>
<tr>
<td>day 21</td>
<td>66/34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 29</td>
<td>80/20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 37</td>
<td>58/42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 47</td>
<td>48/52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 52</td>
<td>47/53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PtR day 9</td>
<td>62/38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 16</td>
<td>32/68</td>
<td></td>
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</table>

X Normal = 14
Table 6

PA Activity of MØ subsets after interaction with patient T cells

<table>
<thead>
<tr>
<th></th>
<th>Unsep&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fc&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Fc&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Unsep + T&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Fc&lt;sup&gt;-&lt;/sup&gt;+T</th>
<th>Fc&lt;sup&gt;+&lt;/sup&gt;+T</th>
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<tbody>
<tr>
<td>Pt</td>
<td>43.8</td>
<td>32.9</td>
<td>59.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nor</td>
<td>49.6</td>
<td>29.4</td>
<td>41.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pt</td>
<td>34.1</td>
<td>38.3</td>
<td>41.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nor</td>
<td>26.1</td>
<td>27.0</td>
<td>41.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pt</td>
<td>10.9</td>
<td>8.2</td>
<td>19.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nor</td>
<td>19.3</td>
<td>11.5</td>
<td>29.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Pt</td>
<td>19.4</td>
<td>22.6</td>
<td>31.5</td>
<td>-</td>
<td>29</td>
<td>25.2</td>
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<tr>
<td>Nor</td>
<td>21.7</td>
<td>22.4</td>
<td>33.5</td>
<td>-</td>
<td>29</td>
<td>30.1</td>
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<tr>
<td>Pt</td>
<td>31.7</td>
<td>20.6</td>
<td>46.4</td>
<td>18.6</td>
<td>29.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Nor</td>
<td>26.5</td>
<td>21.4</td>
<td>35.4</td>
<td>-</td>
<td>-</td>
<td>19.2</td>
</tr>
</tbody>
</table>

a. Plasmin specific fibrinolysis of 5 x 10<sup>5</sup> unseparated (Unsep) heterogenous MØ from either patients (Pt) or Normals (Nor)

b. 5 x 10<sup>5</sup> MØ with high affinity receptors for IgG rosetting with anti-RH antibody coated human erythrocytes

c. 5 x 10<sup>5</sup> MØ depletion of Fc<sup>+</sup> rosetting cells

d. 5 x 10<sup>5</sup> MØ derived from cultures incubated with Pt T lymphocytes collected at 3-4 days after the MØ
Table 7

| FC⁺ MO Subset Containing the MO which are Producing Leukocyte Pyrogen (LP) |
|----------------|----------------|
| MO Popᵃ       | Patient       | Normal       |
|                | PGE₂  | LPᵇ         | PGE₂  | LP |
| FC⁺            | 12.665 | .55         | 4.058 | .25 |
| FC⁻            | 1.484  | 0           | 847   | 0  |

A. MO populations (Pop) were selected for FC receptor by rosetting with anti RH coated red cells.

B. Leukocyte pyrogen was assayed as change in temperature (Δ Temp) of mice injected with 0.3 mL of MO supernate.
A. T lymphocytes were isolated from patient mononuclear preparations by E rosetting of MΦ depleted populations

B. 2x10^6 normal MΦ were cocultured for 2 days with 2x10^6 patient T lymphocytes

FIG. 1

Effect of patients' T cells on normal MΦ functions
A. Isolation of patient Fc⁺ and Fc⁻ Mo populations performed by rosetting with anti-Rh antibody coated human erythrocytes

B. Non rosetting population

C. Percent of the total population rosetting with anti-Rh coated erythrocytes

FIG. 2

Increase in Fc⁺ Mo population in immunoincompetent patients
**FIG. 3**

*Fc*⁺ Mφ subset a contains the PGE₂ producing cells
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