The Role of Prostaglandin E_2 in Immune Suppression Following Injury

JOHN T. GRBIC, D.M.D., JOHN A. MANNICK, M.D., DAVID B. GOUGH, F.R.C.S.I., M.Ch., and MARY L. RODRICK, Ph.D.

It has been thought for some time that prostaglandin E_2 (PGE_2) released from activated monocytes/macrophages may contribute to the suppression of immunity seen after burns and major injury because PGE_2 inhibits the activation of T lymphocytes. To clarify this issue, we studied 15 patients with total body surface area burns of 20% to 90% (mean, 48%). Peripheral blood mononuclear cells (PBMC) were obtained from these patients one to two times each week for 1 month after burn and were stimulated with the T-cell mitogen phytohemagglutinin (PHA). On 14 occasions the PBMCs from eight patients were significantly suppressed (30% or more) in their response to PHA (suppressed [sup] burn) as compared with PBMCs from normal controls. In 38 instances PBMCs from 12 patients were not significantly suppressed in PHA (nonsupressed [nonsup] burn). Sup burn PBMCs and control PBMCs were cultured with or without the addition of the cyclooxygenase (CO) inhibitor indomethacin (Indo, 1 μg/mL) and studied for PHA response and the production of the stimulatory cytokine interleukin-2 (IL-2). Indo partially restored the PHA response of sup burn PBMCs to normal. Sup burn PBMCs also were deficient in production of IL-2. Indo increased IL-2 production by sup burn PBMCs significantly more (160% ± 20%, $p < 0.005$) than control (57% ± 5%) and nonsup PBMCs (67% ± 8%). Next inhibition of the PHA response of PBMCs from 12 burn patients and 17 controls was studied by exogenous PGE_2. At all time periods after burn injury, patients' PBMCs were significantly more sensitive to inhibition by PGE_2 (50% inhibition at 10^{-8} mol/L [molar] PGE_2) than PBMCs from normal controls (50% inhibition at 10^{-6} mol/L PGE_2) with maximum sensitivity occurring 8 to 14 days after injury. Peripheral blood mononuclear cells from patients with more than 40% burns were significantly ($p < 0.05$) more sensitive to PGE_2 than those from patients with lesser burns. Interleukin-2 was added to cultures of sup burn PBMC, nonsup burn PBMC, and controls containing 10^{-7} mol/L PGE_2. Interleukin-2 totally reversed PGE_2 inhibition of the PHA response in PBMC from both controls and burn patients. Because endotoxin leak from the gut has been implicated as a trigger for a number of the metabolic and immunologic abnormalities following injury, the authors looked for the effect of a bolus infusion of *Escherichia coli* endotoxin (Endo, 4 ng/kg) in seven normal healthy volunteers on the response of PBMC to PHA and on the production of PGE_2 and IL-2. The PHA response and IL-2 production were both significantly ($p < 0.01$) suppressed 4 hours after Endo, returning to normal by 24 hours. At 4 hours PBMCs were also significantly more sensitive ($p < 0.001$) to exogenous PGE_2. Production of PGE_2 by adherent PBMCs was maintained at normal levels at 4 hours and increased markedly by 24 hours after Endo. The CO inhibitor ibuprofen (800 mg 2 hours before and at the time of Endo infusion) prevented a significant decrease in PHA response and IL-2 production by PBMCs and blunted the increased PGE_2 production 24 hours after Endo. The *in vitro* addition of Indo partially and exogenous IL-2 completely restored the PHA response to normal. Exogenous IL-1 had no effect when added to cultures of PBMCs from Endo-treated volunteers. It is concluded that (1) PGE_2 has a role in the suppression of immunity after burn injury; (2) PGE_2 exerts its suppressive effect principally by inhibition of lymphocyte IL-2 production; (3) endotoxin mimics the effect of injury on PGE_2 production, T-cell activation, and IL-2 production; (4) administration of CO inhibitors is likely to be essential for the success of clinical regimens designed to correct the immune suppression that follows major injury.

From the Department of Surgery, Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts

Presented at the 111th Annual Meeting of the American Surgical Association, April 11–13, 1991, Boca Raton, Florida.

Supported by National Institutes of Health grants GM 5R01GM35633-06 and GM 5P50GM365428-06 and by the Brigham Surgical Group Foundation.

Dr. Grbic's present address is Oral Medicine & Pathology, Columbia University College of Dentistry, New York, NY 10032.

Dr. Gough's present address is University College Hospital, Galway, Ireland.

Address reprint requests to Mary Rodrick, Department of Surgery, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115.

Address correspondence to John A. Mannick, Department of Surgery, Brigham and Women's Hosptial, 75 Francis St., Boston, MA 02115.

Accepted for publication April 22, 1991.

DEPRESSION OF CELL-MEDIATED immunity in patients following thermal or traumatic injury has been demonstrated by anergy to recall antigens,[1] delayed allograft rejection,[2] and decreased response to T-cell mitogens.[3] Our laboratory[4,5] has shown that a primary immunologic defect in patients following

severe injury was the decreased production of the lymphokine interleukin-2 (IL-2).

The arachidonic acid metabolite, prostaglandin E_2 (PGE_2) has been shown to decrease T-cell activation by inhibiting the production of IL-2.[6] The ability of PGE_2 to inhibit IL-2 production is believed by some investigators to be due to the activation of a suppressor T cell,[7] although this cell, if it exists, has never been well characterized. A more likely explanation of PGE_2 action on T lymphocytes is its well-defined ability to stimulate adenylate cyclase activity and thus to increase intracellular cyclic adenosine monophosphate (cAMP) concentration, a negative signal for T-cell activation. In burn patients, increased levels of PGE_2 have been found in the area of the burn wound[8] and in the adjacent lymphatics[9] and in serum.[8] Antonacci et al.[10] reported an increased conversion of arachidonic acid to PGE_2 by monocytes obtained from burn patients. Wood et al.[11] have shown that the cyclooxygenase inhibitors indomethacin (Indo) and flurbiprofen could increase *in vitro* IL-2 production by splenocytes from thermally injured mice and Faist et al.[12] were able to increase the mitogen responsiveness of circulating lymphocytes from patients with traumatic injury by the addition of Indo.

In this study we examined the role of PGE_2 in the inhibition of IL-2 production in burn patients by determining (1) the effect of the cyclooxygenase inhibitor Indo on the mitogen responses and IL-2 production of peripheral blood mononuclear cells (PBMC) from these patients, (2) whether there was a difference in sensitivity of PBMC to PGE_2 when burn patients were compared with controls, and (3) the effect of the addition of IL-2 to PGE_2-suppressed mitogen responses.

There is a burgeoning consensus that the trigger for many of the immunologic abnormalities noted after traumatic or thermal injury is bacterial endotoxin,[13] either leaked directly from the gut into the portal circulation or produced by intestinal bacteria translocated from the gut in response to injury. Because bacterial endotoxins are well-known stimuli of monocyte and macrophage PGE_2 production, we also studied the effect of the administration of small doses of bacterial endotoxin to normal human volunteers to determine the effect of endotoxin on T-cell mitogen responses and IL-2 production and on the production of PGE_2 by circulating monocytes.

Materials and Methods

Human Subjects

After obtaining informed consent, 15 patients were studied after admission to the Burn Unit of the Brigham & Women's Hospital. Their ages ranged from 18 to 65 years, with a mean age of 35 years. The mean burn size was 48% total body surface area (TBSA), with a range of 20% to 90%. The study was approved by the Committee for the Protection of Human Subjects from Research Risks of the Brigham & Women's Hospital. Venous blood samples were obtained from the patients one to two times weekly until their discharge from the Burn Unit or until their death. Eight of the fifteen patients studied had at least one septic episode as defined by positive blood cultures and/or strong clinical evidence that prompted a course of antibiotics. One of the patients died following the development of sepsis. Thirty normal healthy individuals were used as controls and were age and sex matched whenever possible.

The volunteers for the endotoxin study were 13 normal men (mean age, 33 years; range, 28 to 39 years), who were judged healthy by history, physical examination, hematology, chemical studies, and stress electrocardiogram. They were admitted 4 days before the study to the Clinical Research Center (CRC) of the Brigham & Women's Hospital, Boston, Massachusetts for acclimatization and evaluation. Written informed consent, which was reviewed and approved by the Brigham & Women's Hospital Committee for Protection of Human Subjects from Research Risks, was obtained for all volunteers. The subjects were given a standard hospital diet and activity was limited to the Clinical Research Center. Ambulatory, normal healthy laboratory volunteers were also studied at the same times as infused hospitalized volunteers. Two normal subjects of similar age were studied for each hospitalized volunteer.

Seven volunteers in separate hospitalizations were given either an intravenous bolus infusion of purified *Escherichia coli* endotoxin (4 ng/kg) in saline into an indwelling intravenous catheter or an infusion of saline alone. Venous blood samples were drawn from the volunteers before endotoxin or saline infusion (0 hours) and at 4 and 24 hours after infusion. Six other volunteers received two doses of ibuprofen, 800 mg orally, 2 hours before and at the time of Endo or saline infusion.

Reagents

Roswell Park Memorial Institute (RPMI) 1640 medium, minimum essential medium (Eagle) (MEM), l-glutamine, n-2 hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), antibiotic-antimycotic solution (5000 units penicillin/5000 μg streptomycin/25 μg Fungizone/mL) (PS), and fetal bovine serum (FBS) were obtained from Grand Island Biological Co. (Grand Island, NY). Beta-2-mercaptoethanol (2-ME) was obtained from Eastman Kodak (Rochester, NY). Fetal bovine serum was heat inactivated at 56 C for 30 minutes and filtered through 0.45-μm Nalgene filters (Nalgene Co., Rochester, NY) before use. Purified phytohemagglutinin (PHA) was a gift of Dr. Peter Polgar. Tritiated thymidine (^{3}HTdr, 6.7 Ci/mmol/L [millimolar]) was obtained from New England

Nuclear (Boston, MA). Purified human interleukin-2 (Lymphocult-TLF) was obtained from Biotest (FRG) (400 units/mL). Recombinant human IL-1 and IL-2 were purchased from Genzyme Corp. (Boston, MA). Indomethacin and PGE_2 were obtained from Sigma Chemical Co. (St. Louis, MO). The Indo and PGE_2 were dissolved in 70% ethanol at concentrations of 2 mg/mL and 1 mg/mL, respectively. These were diluted to their final concentration in complete medium (described below).

Preparation of PBMCs

Peripheral blood mononuclear cells were harvested by centrifugation of heparinized peripheral blood diluted 1:2 in phosphate-buffered saline on Ficoll-Hypaque (Pharmacia, Piscataway, NJ) for 35 minutes at 400*g*. The interface cells were collected and washed three times in MEM with 1% PS, 2 mmol/L glutamine and 1% HEPES (MEM-PS). After the third wash, the cells were placed in MEM-PS containing 5% FBS (complete medium, CM) and counted using trypan blue for viability and Turk's stain for observation of nuclear morphology. Cells were always more than 95% viable. As patients' Ficoll interface cells often have increased contamination with myeloid cells, estimations of mononuclear cells by Turk's stain were made and cell counts adjusted accordingly so the total number of mononuclear cells per well of all cultures was similar. Peripheral blood mononuclear cells were collected from one or more patients or Endo-treated volunteers and two untreated volunteers for each day's tests.

Blastogenesis Assay and the Effect of Indomethacin

Peripheral blood mononuclear cells isolated as above were cultured in 220 μL/well volume at 1×10^5 cells/well in 96-well flat-bottomed tissue culture plates (Nunc, Denmark) containing CM. The T-cell mitogen PHA was added at a concentration of 6 μg/ml for 90 hours at 37 C in 5% CO_2 and the wells were pulsed with 1 μCi ^{3}HTdr during the last 18 hours of culture. Indo (1 μg/mL) was added to some of the wells at the initiation of the assay. The cells were harvested on a multiple automated sample harvester (Cambridge Technology, Cambridge, MA) and the incorporated radioactivity was measured by liquid scintillation counting in an LKB beta counter (LKB Instruments Inc., Gaithersburg, MD). The mean amount of radioactivity was determined from triplicate cultures and expressed in counts per minute. The standard deviation of the mean of triplicates never exceeded 10%. The counts per minute (cpm) of the cultures containing no mitogen (background cpm) were subtracted from the cultures with mitogen and this number was used in all calculations. Patients and normal volunteers were compared and, to avoid interassay variation, suppression of the lymphocyte response was calculated by the formula:

$$\%\ \text{Suppression} = 1 - \frac{\text{patient cpm}}{\text{control cpm}} \times 100$$

In addition the percentage enhancement with Indo was calculated for each patient and normal control by the following formula:

$$\%\ \text{Enhancement} = \left[\frac{\text{cpm with PHA + Indo}}{\text{cpm with PHA}} - 1\right] \times 100$$

Determination of PBMC Sensitivity to PGE_2

The sensitivity of PBMCs to the inhibitory effects of PGE_2 was determined by the method of Goodwin et al.[13] A total of 1×10^5 PBMCs were placed into culture with 200 μL of CM containing 1 μg/mL of Indo (to prevent *de novo* PGE_2 synthesis), and either 0, 10^{-6}, 10^{-7}, or 10^{-8} mol/L (molar) PGE_2. Twenty microliters of the T-cell mitogen PHA (final concentration: 6 μg/mL) was added, the cultures were incubated, pulsed, and harvested, and incorporated radioactivity determined as mentioned above. The percentage suppression for each concentration of PGE_2 was determined by the formula:

$$\%\ \text{Suppression} = 1 - \frac{\text{cpm of cultures with PGE}_2 + \text{Indo}}{\text{cpm of cultures with Indo only (0 M PGE}_2)} \times 100$$

The dose of PGE_2 that caused 50% inhibition (ID_{50}) was calculated by determining the two PGE_2 concentrations more than and less than 50% inhibition and extrapolating from these two points the concentration of PGE_2 that caused 50% inhibition. If the 50% inhibition was more than 1×10^{-6} mol/L or less than 1×10^{-8} mol/L, then the ID_{50} was set at 1×10^{-5} mol/L and 1×10^{-9} mol/L PGE_2, respectively.

Addition of Purified IL-2 to PGE_2 Containing Mitogen Cultures

Peripheral blood mononuclear cells were cultured at a concentration of 1×10^5 cells/well as above with 200 μL of CM containing 1 μg/mL Indo, 10^{-7} mol/L PGE_2, and 6 μg/mL PHA. Twenty microliters of ultrapurified human natural IL-2 (final concentration: 8 units/mL) or the same volume of CM were added to the wells. The cells were incubated, pulsed, harvested, and the incorporated radioactivity determined as above. The percentage suppression compared to Indo alone was calculated as above.

Production of PGE_2 by Adherent Cells

Peripheral blood mononuclear cells (200 μL/well) in CM at a concentration of 5×10^6/mL were incubated in

96-well microliter plates for 1 hour at 37 C. Nonadherent cells were removed by washing with CM. Adherent cells were then cultured in FBS-free RPMI (with antibiotics, HEPES, and glutamine as above) for 24 hours with or without the addition of *E. coli* 055B5 lipopolysaccharide (Sigma Chemical Co., St. Louis, MO) at a concentration of 1.5 μg/mL. Supernantants were harvested and the PGE_2 concentration was measured by commercial radioimmunoassay (Advanced Magnetics, Cambridge, MA).

Production of IL-2

Interleukin-2 was generated by culturing PBMCs at 1 $\times 10^5$ cells/well in 200 μL volume for 24 hours in CM with or without 2.5 μg PHA/well. Indo was added to some of the cultures at a final concentration of 1 μg/mL. Supernatants were collected and frozen at −70 C until assayed.

IL-2 Assay

Assay of IL-2 was made as previously described.[5] Supernatants to be tested for IL-2 were diluted from 1:2 to 1:64 in medium in 100 μL volume as above and incubated for 1 hour at 37 C, 5% CO_2. CTLL-2 cells were freed of T-cell growth factor (TCGF) by washing three times (800 rpm for 7 minutes) in RPMI 1640 medium containing 1% PS, 1% HEPES, 5% FBS, 2 mmol/L glutamine, and 5 $\times 10^{-5}$ mol/L 2-ME. The cells were then diluted to 4 $\times 10^4$/mL and 100 μL added to each well. Cultures were incubated for 20 hours at 37 C in 5% CO_2. One μCi 3HTdr/well was added, cultures were harvested 4 hours later, and then counted as above. Units of IL-2 were determined by comparison with a standard TCGF prepared as previously described.[5] The IL-2 content was calculated using a program provided by Dr. Brian Davis (Immunex Corp., Seattle, WA) on a Kaypro 2 microcomputer. T-cell growth factor was assigned a value of 1 unit.

Statistical Analysis

Statistical analyses were performed using a Vax 11/780 computer (Digital Equipment Corp., Waltham, MA) and a standard statistical package (Minitab, University of Pennsylvania, Philadelphia, PA). A normal probability plot was used to detect nonparametric data and the appropriate statistical methods (Student's t test for parametric data and Mann-Whitney U test for nonparametric data) were used.

Results

Enhancement of the Mitogen Response by Indo

To determine whether PGE_2 played a role in suppressing the PHA-induced mitogen responses of burn patients, we studied the effect of the cyclooxygenase inhibitor Indo on the mitogen responses of these patients. The patients' PBMC PHA response data were separated into two groups: (1) those with a mitogen response suppressed 30% or more (n = 14 determinations in 8 patients) compared with controls (sup burn) and (2) those with mitogen responses suppressed less than 30% (n = 38 determinations in 12 patients) (non sup burn). The 30% level was considered to be significant in our laboratory because none of the normal controls were suppressed 30% or more from the mean normal value and this value was calculated to be more than two standard deviations from mean of the normal controls. Our results (Fig. 1) indicate that PBMCs from normal controls (n = 65 determinations) and the nonsup burn patient cultures were enhanced 12% ± 3% and 13% ± 3% (mean ± standard error of the mean [SEM]) by Indo, respectively. The mitogen responses of the sup burn PBMCs were enhanced significantly more, 43% ± 5% (mean ± SEM) ($p < 0.001$), by the addition of Indo when compared to normal controls and nonsup burn PBMCs. However the responses of the sup burn PBMCs were not returned to the level of the normal controls by Indo. The mean suppression observed in the sup burn group was 49% and after the addition of Indo, the suppression decreased to a mean of 28%.

Enhancement of IL-2 Production by Indomethacin

Because PGE_2 has been shown to function by inhibiting the production of IL-2,[6] we examined the effect of Indo

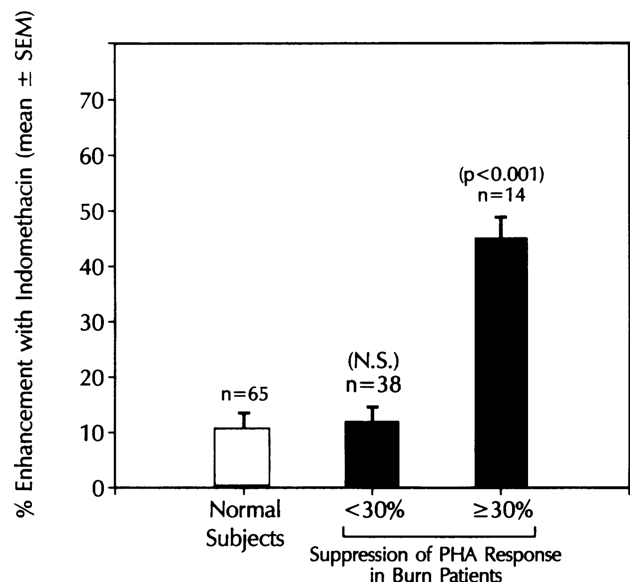

FIG. 1. Ability of indomethacin to enhance the PHA response of burn patients' PBMCs. Indomethacin significantly enhanced ($p < 0.001$) the PHA-induced blastogenesis of sup burn PBMC (see text) when compared to PBMCs from both normal controls and the nonsup burn groups (n = the number of determinations in each group). NS, not significant.

on the IL-2 production of burn patients' PBMCs. The cell cultures were separated again into two groups: (1) cells from patients with an IL-2 production that was suppressed 50% or more compared to the mean of the normal controls (sup burn) (n = 28 determinations in 10 patients) and (2) cells from patients with an IL-2 production that was less than 50% suppressed (nonsup burn) (n = 39 determinations in 14 patients). The 50% suppression level was considered significant in our laboratory because none of normal controls was found to have his/her IL-2 production suppressed more than 50% and this value was approximately two standard deviations from the mean of the normal controls. The patients were compared to 15 normal controls (mean age, 32 years) whose mean 24-hour IL-2 production was 1.46 ± 0.11 units (mean ± SEM). The results (Fig. 2) demonstrate that PBMC from all three groups exhibited increased IL-2 production with the addition of Indo. However the sup burn group was significantly more enhanced (160% ± 20%) (mean ± SEM) ($p < 0.0005$) than both the normal control (57% ± 5%) and nonsup burn (67% ± 8%) groups. The IL-2 production of the sup burn group was 0.37 units, which was enhanced to 0.96 units with the addition of Indo; however this remained lower than the level of normal controls (1.46 units).

It has been reported that PGE_2 may affect the IL-2 assay by suppressing the proliferation of the IL-2–dependent cell line directly,[14] although this finding has been disputed

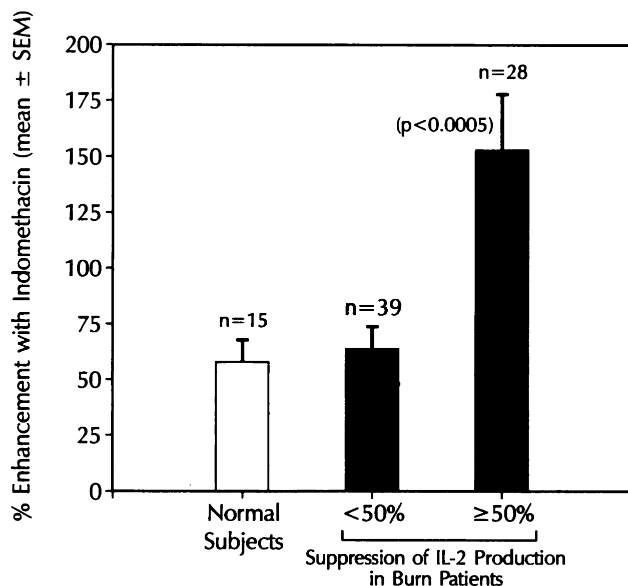

FIG. 2. Enhancement of IL-2 production by indomethacin in burn patients. Burn patients' PBMCs were divided into a nonsuppressed group (see text) and a suppressed group and compared with normal controls. In all three groups, IL-2 production by PBMC was enhanced by indomethacin, although the sup burn group was significantly ($p < 0.0005$) more enhanced than either the normal controls or the nonsup group (n = the number of determinations).

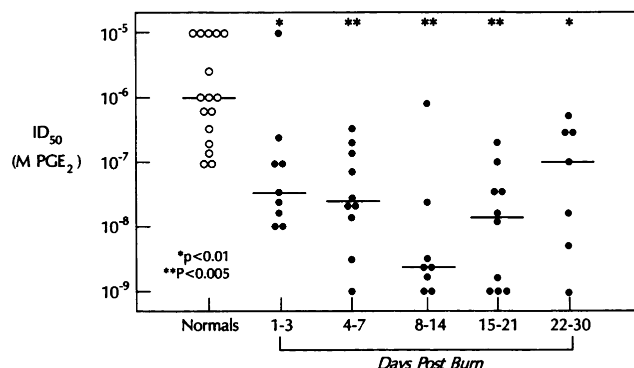

FIG. 3. Increased sensitivity by PBMC to PGE_2 after thermal injury. The ID_{50} concentration of PGE_2 was determined for normal controls (○) and burn patients (●) up to 30 days after injury. Horizontal lines in each group represent the median value of that group. Burn patients' PBMCs were found to be significantly more sensitive to PGE_2 than normal controls at every time point up to 30 days. Maximal sensitivity appeared to be at 8 to 14 days after injury.

by other investigators.[7,8] We tested the proliferation of the CTLL-2 cells by adding concentrations of PGE_2 normally found in cultures of PBMCs (10^{-7} mol/L to 10^{-8} mol/L) with the standard preparation of TCGF in several assays and found no significant effect of PGE_2 on these cells directly (10^{-7} mol/L = 0.92 units; 10^{-8} mol/L = 0.93 units; 0 mol/L = 1.00 units). In addition Indo had no direct effect on the proliferation of the CTLL-2 cell line. Therefore these findings suggest that a metabolite of the cyclooxygenase pathway, presumably PGE_2, inhibited IL-2 production by PBMCs of burn patients.

Increased Sensitivity of Burn Patients' PBMCs to Inhibition by PGE_2

To investigate further the role of PGE_2 in the immunosuppression of burn patients, we determined the sensitivity of their PBMCs to inhibition by PGE_2 in the presence of Indo, as described above. In this study we examined 12 burn patients (mean age, 35 years; range, 22 to 65 years; TBSA, 45%; range, 20% to 90%) and compared them to 17 normal controls (mean age, 32 years; range, 22 to 61 years). The data are illustrated in Figure 3. The results indicate that at all time periods, the patients' PBMCs were significantly more sensitive to inhibition by PGE_2 than those of normal controls, with the maximum sensitivity occurring 8 to 14 days post burn. We also examined the relationship between burn size and PGE_2 sensitivity and found that PMBCs from patients with 40% or more TBSA burns were significantly ($p < 0.05$) more sensitive to the inhibitory effects of PGE_2 than those from patients with less than 40% TBSA burns (Fig. 4). Because the patients who developed clinically evident sepsis were also the patients with the biggest burns, it was difficult to determine which of these factors, burn size or sepsis, was

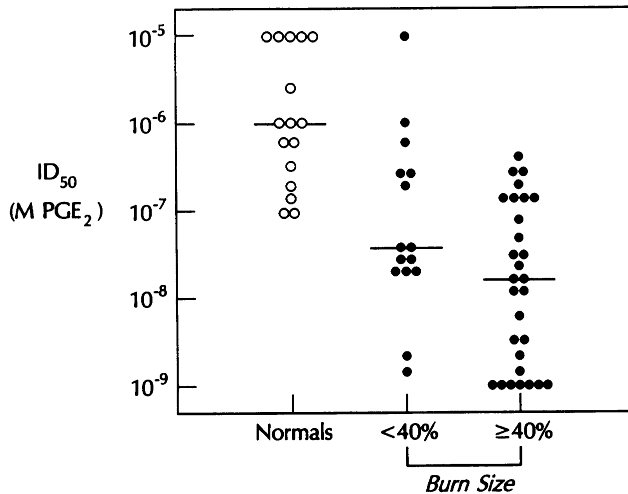

FIG. 4. Sensitivity to PGE_2 of PBMCs from patients with large *versus* small burns. Patients were divided into two groups: those with burns of 40% or more TSBA and those with less than 40% TBSA burns. The horizontal line indicates the median for each group. PBMCs from patients with 40% or greater TBSA burns were significantly ($p < 0.05$) more sensitive to PGE_2 than those patients with less than 40% TBSA burns. Both patient groups were significantly more sensitive to PGE_2 than normal controls.

more important in determining the PGE_2 sensitivity of the PBMCs from burn patients.

We also examined the relationship between suppressed mitogen responses and increased sensitivity to PGE_2. Prostaglandin E_2 sensitivity data were available on 13 patients' PBMCs whose PHA responses were suppressed more than 30% as compared to normal controls. Nine of these thirteen patients had ID_{50} values of 10^{-8} mol/L PGE_2 and the remaining four all had ID_{50} values of 10^{-7} mol/L PGE_2. These findings demonstrate that the increased sensitivity to PGE_2 may be an important cause of the suppression of mitogen responses seen in burn patients. However no direct correlation between suppression of the mitogen response and sensitivity to PGE_2 was found in PBMCs from burn patients ($r = 0.17$) or normal controls ($r = 0.01$).

Abrogation of PGE_2-mediated Suppression by IL-2

To determine whether PGE_2 functioned by inhibiting IL-2 production, we attempted to reverse the suppression of the mitogen response caused by PGE_2 by the addition of IL-2. A supraoptimal dose of purified human IL-2 (8 units/mL) was added to PBMC cultures that contained 10^{-7} mol/L PGE_2, 1 μg/mL Indo (to block *de novo* synthesis of PGE_2), and 6 μg/mL PHA, and these cells were assayed for mitogenic activity. The responses of the cultures containing PGE_2 with or without IL-2 were compared to those of cultures containing Indo and PHA only. The data are presented in Figure 5. Peripheral blood mononuclear cells from patients were significantly more suppressed by PGE_2 ($p < 0.005$) when compared to normal controls, again illustrating the increased sensitivity of burn patients' PBMCs to PGE_2. With the addition of IL-2, the PGE_2-mediated inhibition of the mitogen response in both normal controls and burn patients was totally reversed. This suggests that PGE_2-mediated suppression of lymphocyte activation in burn patients results primarily from the inhibition of IL-2 production by PGE_2.

PHA Responsiveness and IL-2 Production by PBMC from Endo-treated Volunteers

Volunteers were studied before (0 hours) receiving an intravenous infusion of *E. coli* endotoxin (4 ng/kg) and at 4 and 24 hours after Endo infusion. Peripheral blood mononuclear cells were harvested and studied for their responsiveness to PHA and the ability to produce IL-2 in response to PHA stimulation. Endo-treated volunteers were compared with those receiving infusions of saline alone. As noted in Figure 6, 4 hours after Endo infusion there was a significant inhibition of the ability of treated individuals' PBMCs to respond to PHA. This was accompanied by a simultaneous inhibition of production of IL-2 by PBMCs in response to PHA. Both these effects had returned to normal by 24 hours after Endo infusion.

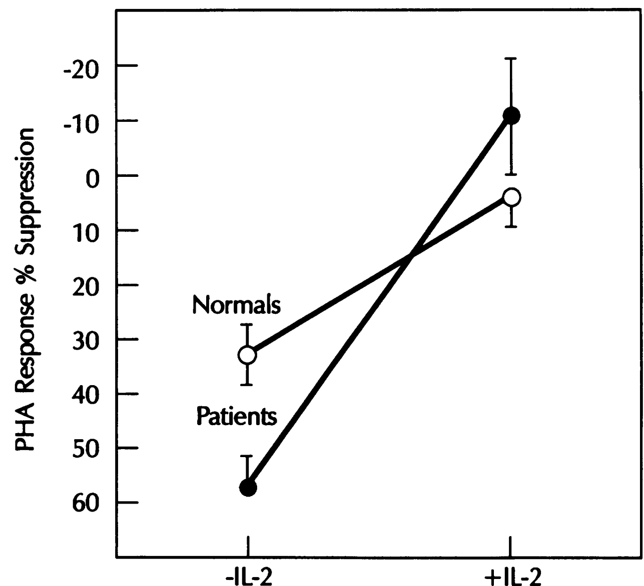

FIG. 5. The effect of the addition of IL-2 to cultures of burn patient PBMCs. Purified IL-2 was added to a PHA-induced blastogenesis assay that contained 10^{-7} mol/L PGE_2 and Indo. The vertical axis represents the percentage of the mitogen response in comparison to cultures that contained Indo only (0% suppression). The open circles represent the cultures of normal PBMCs and the closed circles represent PBMCs from patients. The values are the mean ± SEM.

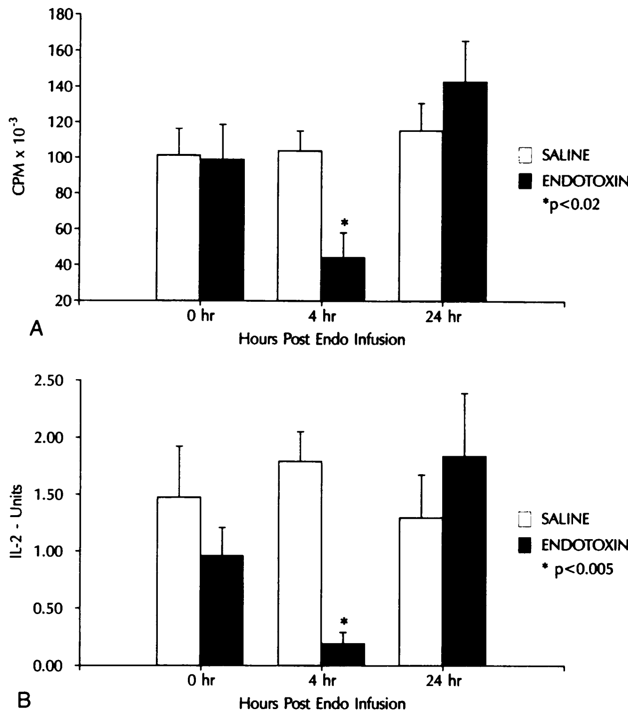

FIG. 6A and 6B. Effect of endotoxin infusion on PHA response and IL-2 production. PBMCs were harvested from volunteers treated with saline (■) or Endo (□) and studied for (A) PHA response and (B) IL-2 production. Four hours after Endo infusion there was a significant ($p < 0.02$) inhibition of the PHA response ($p < 0.002$) and IL-2 production ($p < 0.005$), which returned to normal by 24 hours.

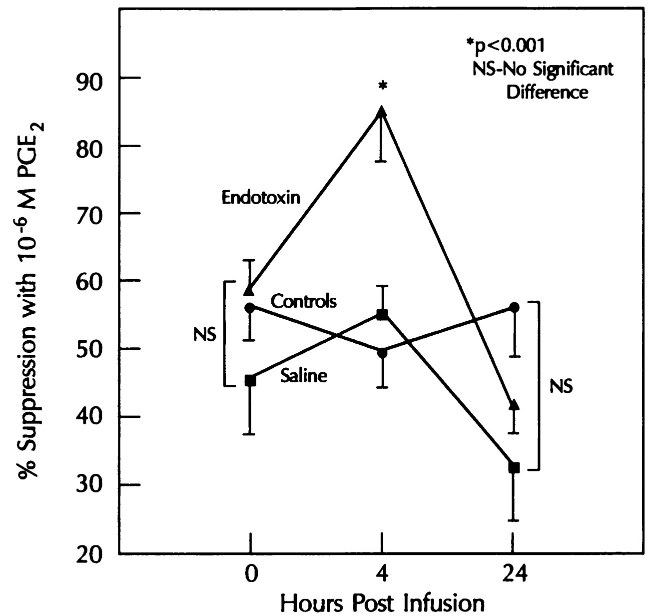

FIG. 7. Effect of Endotoxin infusion on the sensitivity of PBMC to PGE_2. PBMCs were harvested from volunteers treated with saline (■), endotoxin (▲), and normal controls (●) and studied for their inhibition by PGE_2 during response to PHA. PBMC from Endo-treated volunteers were more sensitive to inhibition by PGE_2 4 hours after Endo infusion ($p < 0.001$).

Sensitivity of PBMC from Endo-treated Volunteers to PGE_2

To determine the effect of Endo infusion on the sensitivity of PBMCs to exogenous PGE_2, PBMCs from Endo-treated volunteers were studied for their sensitivity to inhibition by graded dosages of PGE_2 in their responsiveness to PHA in experiments similar to those reported above for burn patients. Again (Fig. 7) in these experiments PBMCs from Endo-treated volunteers were markedly more sensitive to inhibition *in vitro* by PGE_2 4 hours after Endo infusion. Saline infusion alone produced no such effect.

The Effect of Indo and IL-2 on the PHA Response of PBMCs from Endo-treated Volunteers

To determine whether Indo or exogenous IL-2 could restore the proliferative response of PBMCs to PHA, PBMCs obtained 4 hours after Endo infusion were cultured with PHA with or without the addition of Indo (1 μg/mL) or a supraoptimal dose (10 μ/mL) of recombinant human IL-2 (rhIL-2). The results shown in Figure 8 indicate that Indo partially and IL-2 totally restored the proliferative response to PHA of PBMCs harvested 4 hours after Endo infusion. The addition of exogenous rhIL-1 had no effect.

Effect of Endo Infusion on PGE_2 Production by Adherent Cells from the PBMC Population

Peripheral blood mononuclear cells obtained from Endo- or saline-treated volunteers were allowed to adhere to plastic for 1 hour. Nonadherent cells removed by washing the adherent cells were stimulated in serum-free medium with *E. coli* endotoxin and the production of PGE_2 was measured by determining PGE_2 concentration in the culture supernatant by radioimmunoassay. The results shown in Table 1 indicate that PGE_2 production was maintained early after Endo infusion and had increased

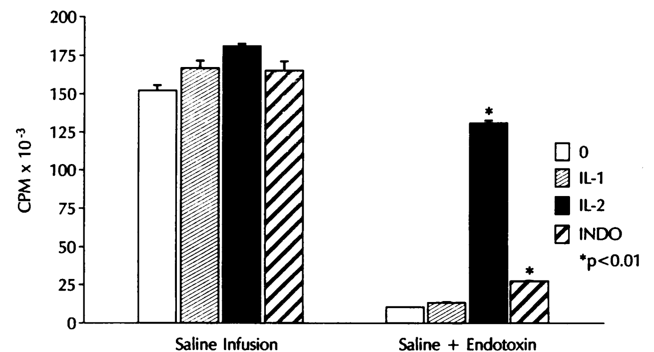

FIG. 8. Effect of indomethacin, IL-2, and IL-1 on the PHA response after endotoxin. PBMCs obtained 4 hours after saline or endotoxin infusion were cultured with PHA with or without the addition of indomethacin, IL-2, or IL-1. Results are shown for one representative experiment of six. Indomethacin partly and IL-2 completely restored the PHA response. IL-1 had no effect. * = $p < 0.01$, compared with 0 addition.

TABLE 1. PGE_2 *Synthesis*

	Infusion 0 (hours)	Time After Infusion (hours) 4	24
Saline	5656 + 1845	2679 + 621	3471 + 525
Endotoxin	5043 + 641	4364 + 1423	9257 + 2857*
Ibuprofen +Saline	5123 + 2376	4654 + 1451	3717 + 781
Ibuprofen +Endotoxin	6094 + 2567	3750 + 812	6975 + 782*

PGE_2 was determined in supernatants of LPS-stimulated adherent PBMC from normal volunteers at indicated times following infusion of saline, endotoxin, saline plus ibuprofen or ibuprofen + endotoxin.
* $p < 0.05$ compared with control infusion at same time.

to supranormal levels 24 hours later. Saline infusion produced no such effect.

Effect of Ibuprofen In Vivo *on PBMC Responses of Endo-treated Volunteers*

In a final series of experiments, six volunteers received 800 mg ibuprofen orally 2 hours before and at the time of infusion with Endo or saline. Peripheral blood mononuclear cells from these volunteers were tested for PHA response, IL-2 production, and adherent cells for PGE_2 production as above. It can be seen from Figure 9 that ibuprofen pretreatment abrogated the depression of the PHA response of PBMCs following Endo treatment. It also partially (but significantly) corrected the inhibition of IL-2 production following Endo infusion. Finally ibuprofen pretreatment blunted but did not entirely prevent the excessive PGE_2 production by adherent PBMCs noted 24 hours after Endo infusion (Table 1).

Discussion

Our laboratory has previously shown decreased IL-2 production by PBMCs from burned[4] and traumatically injured[5] patients despite normal or increased IL-1 production by adherent mononuclear cells from these patients. Prostaglandin E_2 has been shown to be a potent inhibitor of IL-2 production[6,7] and enhanced production of PGE_2 by monocytes obtained from burned patients has been demonstrated.[10] In addition increased levels of PGE_2 have been reported in both the burn wound and in the adjacent lymphatics,[8,9] as well as in the serum of burn patients.[13] In this study we examined the role of PGE_2 in the decreased T-cell responses and IL-2 production we have observed in burn patients.

Our results using the cyclooxygenase inhibitor indomethacin to enhance depressed mitogen responses and IL-2 production strongly suggest that PGE_2 does play a significant role in modulating the immune responses of PBMCs obtained from burned patients. These findings correlate with those of Faist et al.,[12] who showed that indomethacin was able to enhance the PHA response of PBMCs from patients with traumatic injury who demonstrated a depressed immune function. However indomethacin did not have a significant effect on PBMCs from normal controls or trauma patients who did not exhibit depressed mitogen responses. On the other hand, Antonacci et al.,[10] using the cyclooxygenase inhibitor WY-18251, found no increases in the autologous mixed lymphocyte reaction of burn patients 5 to 7 days after burn, although this work did show that monocytes from the same patients produced greatly increased quantities of PGE_2.

In the present study we have shown that the administration of a single dose of endotoxin to normal human volunteers can reproduce many of the abnormalities of lymphocyte function noted in the burn patients. These include diminished responsiveness of circulating lymphocytes to the T-cell mitogen PHA and diminished production of IL-2, in response to PHA stimulation. We also noted increased production of PGE_2 by adherent cells in the PBMC population. These findings lend further credence to the idea that many of the abnormalities in immune responsiveness that accompany serious injury are triggered by bacterial endotoxin leaked from the gut or released from enteric organisms translocated from the gut.

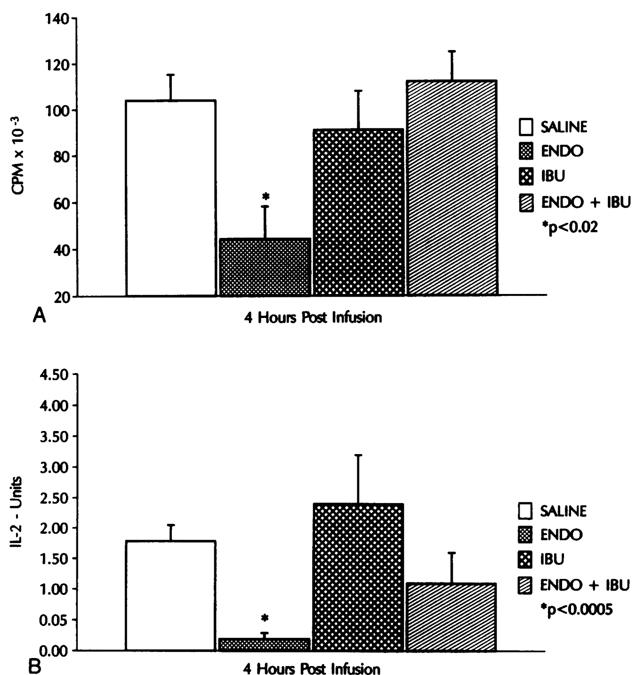

FIG. 9A and 9B. Effect of ibuprofen *in vivo* on PBMC responses after endotoxin. PBMCs obtained from volunteers who received either saline, endotoxin (Endo), or ibuprofen (IBU) before and at the time of saline or endotoxin infusion were studied for (A) PHA response and (B) IL-2 production. Ibuprofen pretreatment abrogated the depression of the PHA response and partially corrected the inhibition of IL-2 production seen after endotoxin.

The fact that many of the abnormalities of PBMC function noted in endotoxin-treated volunteers could be abrogated by ibuprofen further highlights the role of prostanoid production in mediating these abnormalities.

We also demonstrated that PBMCs from burn patients were considerably more sensitive to the inhibitory effects of PGE_2 than PBMCs from normal individuals. Furthermore burn patients' PBMCs, which exhibited suppressed mitogen responses, tended to be most sensitive to the inhibitory effects of PGE_2. The finding of increased sensitivity to PGE_2 supports the work of Goodwin and colleagues,[14] who reported an increased sensitivity to PGE_2 by PBMCs of patients who had undergone cardiac surgery or maternal labor. However their patients' PGE_2 sensitivity reached a peak 1 day after the stress and returned completely to normal within 10 days. They concluded that physical stress could cause an increase in the sensitivity of lymphocytes to PGE_2 and this could account for the immunosuppression seen in these patients.

Our results indicate that lymphocytes from thermally injured patients had a more profound sensitivity to PGE_2 that lasted for a much longer time than those reported by Goodwin et al.[14] and reached a peak between 8 and 14 days after injury. This time period corresponds to the peak incidence of sepsis in our patients and sepsis may have played a role in the increased sensitivity to PGE_2 we observed. Cahill et al.[15] have shown that mice infected with *Salmonella enteriditis* had depressed IL-2 production that was associated with an increased sensitivity of lymphocytes to the inhibitory effects of PGE_2. Furthermore preliminary studies in our laboratory on three uninjured septic patients demonstrated that all of these patients' PBMCs had increased sensitivity to PGE_2.

There are several possible mechanisms for the increased sensitivity of PBMCs to PGE_2. First it may be due to the increased ability of any suppressive substance to inhibit further an already suppressed immune response. Our results show that there was no direct correlation between the amount of suppression seen in both the mitogen response and IL-2 production of normal controls' and burn patients' PBMCs and sensitivity of these PBMCs to PGE_2. Goodwin et al.[14] have also reported similar findings in their study of PGE_2 sensitivity and physical stress, which suggested that increased suppression of mitogen responsiveness does not cause the increased sensitivity to PGE_2. Many of our patients' PBMCs demonstrated an increased sensitivity to PGE_2 although their mitogen responses and IL-2 production were not suppressed.

A second potential cause for increased sensitivity to PGE_2 is an increase in the number of cells capable of responding to PGE_2. Fischer et al.[16] have shown that only 10% to 30% of the peripheral T cells in normal individuals have receptors for PGE_2 and these cells were shown to be responsible for PGE_2-mediated suppression. Presumably an increase in the proportion of the T cells bearing PGE_2 receptors would increase the sensitivity of the total PBMC population to PGE_2. Goodwin et al.[14] have shown an increase in the relative proportion of T cells bearing the receptor for the Fc gamma chain of immunoglobulin in patients following surgery and maternal labor who had demonstrated increased sensitivity to PGE_2. This finding suggested that changes in T-cell subsets could account for some of the increased sensitivity to PGE_2 in their patients. Changes in T-cell subsets, including an increase in T cells bearing the Fc gamma receptor, have been reported in burn patients[17] and, therefore, the possibility exists that these changes may account for the increases in PGE_2 sensitivity seen in our burn patients.

A third possible cause is the presence of serum factors or hormones, which could increase sensitivity to PGE_2. Thermal and traumatic injury as well as sepsis are all known to increase the production of a wide variety of acute-phase proteins and hormones. One class of hormones that has been shown to increase sensitivity of lymphocytes to PGE_2 is the glucocorticoids. Mendelson and colleagues[18] have shown that glucocorticoids were able to enhance the ability of PGE_2 to increase the intracellular production of cAMP in lymphocytes. In 1976 Berenbaum et al.[19] examined the hypothesis that the combination of corticosteroids and PGE_2 could account for the depressed cell-mediated immune responses seen in trauma and burn patients because both were known to be increased after injury. In their system cells from normal individuals were incubated with a wide range of steroid and PGE_2 concentrations. They showed that PGE_2 and corticosteroids were synergistic in their ability to suppress immune responsiveness as assayed by mitogen-induced blastogenesis. Galanaud et al.[20] have shown that lymphocytes removed from volunteers 4 hours after an intravenous injection of hydrocortisone were significantly more sensitive to the inhibitory effects of PGE_2 on the formation of anti-TNP plaques *in vitro*. This suggests that increases in *in vivo* concentrations of hydrocortisone could increase the sensitivity of lymphocytes to *in vitro* PGE_2.

This idea is supported by the present studies of normal volunteers receiving bolus endotoxin infusions. These individuals demonstrated increased PGE_2 production, decreased IL-2 production, and suppressed responsiveness to the T-cell mitogen PHA. These volunteers demonstrated a significant and temporary increase in the sensitivity by circulating PBMCs to inhibition by PGE_2 *in vitro*. As reported elsewhere,[21] endotoxin infusion in these volunteers led to a dramatic increase in serum cortisol levels. This might very well mimic the situation described by Gallanaud et al.[20] and Berembaum et al.[19] and could account for the increased sensitivity of circulating T cells to PGE_2. Pretreatment of the volunteers with ibuprofen was shown to block the *in vivo* increase in serum cortisol

concentrations in addition to inhibiting PGE_2 production, and thus ibuprofen may have abrogated any increased sensitivity of T cells to PGE_2 caused by cortisol.

Our *in vitro* data would tend to support the use of nonsteroidal anti-inflammatory drugs in burn victims and would argue for the addition of such agents to therapeutic trials of biologic agents directed at improving immune responses in seriously injured patients. Others have reached similar conclusions. Hansbrough and colleagues[22] have shown in a burned mouse model that the cyclooxygenase inhibitor, ibuprofen, was capable of restoring a contact sensitivity response to previously anergic animals as well as restoring decreased T helper/T suppressor ratios to normal in the spleens of these animals.[23] Furthermore they demonstrated that ibuprofen was capable of preventing the death of these burned mice following a septic challenge by cecal ligation and puncture.[24] Faist et al.[25] have demonstrated improved cellular immunity in patients receiving indomethacin after major surgery. Results from our laboratory[11] have demonstrated the involvement of PGE_2 in suppressing the production of IL-2 in thermally injured mice and that decreases in IL-2 production are associated with increased deaths following a septic challenge.[26] Furthermore we recently demonstrated that a combination of low-dose IL-2 plus low-dose indomethacin *in vivo* can increase significantly survival in this burn model.[27]

References

1. Wolfe JHN, Wu AVO, O'Connor NE, et al. Anergy, immunosuppressive serum, and impaired lymphocyte blastogenesis in burn patients. Arch Surg 1982; 117:1266–1271.
2. Rapaport FT, Converse JM, Horn L, et al. Altered reactivity to skin homografts in severe thermal injury. Ann Surg 1964; 159:390–395.
3. Daniels JC, Sakai H, Cobb EK, et al. Evaluation of lymphocyte reactivity studies in patients with thermal burns. J Trauma 1971; 11:595–607.
4. Wood JJ, Rodrick ML, O'Mahony JB, et al. Inadequate interleukin 2 production: a fundamental immunologic deficiency in patients with major burns. Ann Surg 1984; 200:311–320.
5. Rodrick ML, Wood JJ, O'Mahony JB, et al. Mechanisms of immunosuppression associated with severe nonthermal traumatic injuries in man: production of interleukin 1 and 2. J Clin Immunol 1986; 6:310.
6. Chouaib S, Fradelizi D. The mechanism of inhibition of interleukin 2 production. J Immunol 1982; 129:2463–2468.
7. Chouaib S, Chatenoud L, Klatzmann D, Fradelizi D. The mechanisms of inhibition of human IL-2 production. II. PGE_2 induction of suppressor T lymphocytes. J Immunol 1984, 132:1951–1857.
8. Arturson MG. Arachadonic acid metabolism and prostaglandin activity following burn injury. *In* Ninneman JL, ed. Traumatic Injury. Baltimore: University Press, 1983, pp 57–79.
9. Anggard E, Arturson G, Jonsson CE. Efflux of prostaglandin in lymph from scalded tissues. Acta Physiol Scand 1970; 80:46(abstr).
10. Antonacci AC, Calvano SE, Reaves LE, et al. Autologous and allogeneic mixed-lymphocyte responses following thermal injury in man: the immunomodulatory effects of interleukin-1, interleukin-1 and prostaglandin inhibitor, WY-18252. Clin Immunol Immunopathol 1984; 30:304–320.
11. Wood JJ, Grbic JT, Rodrick ML, et al. Suppression of interleukin-2 (IL-2) production in an animal model of thermal injury is related to prostaglandin synthesis. Arch Surg 1987; 122:179–184.
12. Faist E, Kupper TS, Baker CC, et al. Depression of cellular immunity after major injury: its association with post traumatic complications and its restoration with immunomodulatory agents. Arch Surg 1986; 121:1000–1005.
13. Deitch AJ, Ma WJ, Ma L, et al. Endotoxin-induced bacterial translocation: a study of mechanisms. Surgery 1989; 106:292–300.
14. Goodwin JS, Bromberg S, Staszak C, et al. Effect of physical stress on sensitivity of lymphocytes to inhibition of prostaglandin E2. J Immunol 1981; 127:518–522.
15. Cahill J, Hopper KE. Immunoregulation by macrophages. III. Prostaglandin E suppresses lymphocyte activation but not macrophage effector function during Salmonella enteriditis infection. Int J Immunopharmacol 1984; 6:9–17.
16. Fischer A, LeDeist F, Durandy A, Griscelli C. Separation of a population of human T lymphocytes that bind prostaglandin E and exert a suppressor activity. U Immunol 1985; 124:815–819.
17. Antonacci AC, Good RA, Gupta S. T-cell subpopulations following thermal injury. Surg Gynecol Obstet 1982; 155:1–8.
18. Mendelson J, Multer MM, Boone RF. Enhancing effects of prostaglandin E1 and dibutyryl cyclic AMP upon lymphocytes in the presence of cortisol. J Clin Invest 1973; 52:2129–2137.
19. Berenbaum MC, Cope WA, Bundrick RV. Synergistic effect of cortisol and prostaglandin E2 on the PHA response. Clin Exp Immunol 1976; 26:534–541.
20. Galanaud P, Crevon M-C, Emilie D, Abaella A. Effect of hydrocortisone on the *in vitro* human antibody response: interaction with monocytes and prostaglandins. Clin Immunol Immunopathol 1983; 29:403–414.
21. Revhaug A, Michie JR, Manson J McK, et al. Inhibition of cyclooxygenase attenuates the metabolic response to endotoxin in humans. Arch Surg 1988; 123:162.
22. Hansbrough J, Peterson V, Zapata-Sirvent R, Claman HN. Postburn immunosuppression in an animal model. II. Restoration of cell-mediated immunity by immunomodulating drugs. Surgery 1984; 95:290–295.
23. Zapata-Sirvent RL, Hansbrough HF. Postburn immunosuppression in an animal model. III. Maintenance of normal splenic helper and suppressor lymphocyte subpopulations by immunomodulating drugs. Surgery 1985; 97:721–727.
24. Zapata-Sirvent RL, Hansbrough JF, Bender EM, et al. Postburn immunosuppression in an animal model. IV. Improved resistance to septic challenge with immunomodulating drugs. Surgery 1986; 99:53–58.
25. Faist E, Ertel W, Cohnert T, et al. Immunoprotective effects of cyclooxygenase inhibition in patients with major surgical trauma. J Trauma 1990; 30:8–17.
26. Moss NM, Gough DB, Jordan AL, et al. Temporal correlation of impaired immune response after thermal injury with susceptibility to infection in a murine model. Surgery 1988; 104:882–887.
27. Horgan PG, Mannick JA, Dubravec DB, Rodrick MD. Effect of low dose recombinant interleukin-1 plus indomethacin on mortality after sepsis in a murine burn model. Br J Surg 1990; 77: 401–404.

Discussion

DR. JONATHAN L. MEAKINS (Montreal, Quebec, Canada): From this excellent study, there are several questions that present themselves, and in the manuscript of over 20 pages I cannot cover all of the issues that come to mind, and so I will address basically three things.

In light of the fact that we have often thought that all patients had defects or at least some degree of the expression of these defects, it seems to me that one of the forward steps taken here is the identification that really only some patients have the full expression of the defect in host defenses. So that although we see that all peripheral blood monocytes