Journal of Clinical Immunology, Vol. 15, No. 2, 1995

Altered IL-10 Levels in Trauma Patients' MØ and T Lymphocytes

CAROL L. MILLER-GRAZIANO,[1,2] ASIT KUMAR DE,[1] and KAREN KODYS[1]

Accepted: November 4, 1994

Trauma results in concomitant immunosuppression and elevated monocyte (MØ) inflammatory cytokine levels. The augmenting or ameliorating effect of IL-10 in septic complications after trauma is controversial. Here, IL-10 levels of trauma patients' and normals' PBMC, isolated MØ, and isolated T cells were assessed and correlated to their PBMC mitogen responses, their T-cell proliferation in an APC independent system, and their MØ production of elevated TNF-α levels. Trauma patients with depressed PBMC responses to PHA stimulation also had significantly decreased IL-10 levels in their stimulated PBMC supernates ($P = 0.0022$) and their MDP-stimulated isolated MØ population ($P = 0.0004$). However, patients with depressed PHA responses could have either normal or depressed T-cell proliferation in an anti-CD3-, anti-CD4-stimulated system. If APC-independent T-cell proliferation was depressed, induced IL-10 levels were suppressed ($P = 0.007$). However, if APC-independent T-cell proliferation was normal or elevated, IL-10 levels could be normal or elevated ($P = 0.018$). Decreased IL-10 levels correlated with depressed mitogen responses and depressed T-cell proliferation. IL-10, therefore, could not be inducing trauma patients' immunosuppression. Patients with elevated MØ TNF-α levels had depressed MØ IL-10 levels.

KEY WORDS: IL-10; trauma patients; monocytes; T cells.

INTRODUCTION

Mortality after severe trauma is often delayed and results from lung or kidney failure rather than from the injury itself (1). Increased, deregulated cytokine production, particularly increases of TNF-α and IL-1, are proposed as mediating some of this posttrauma mortality (1–8). The trauma patients' exaggerated cytokine production has been attributed to repeated bacterial stimulation during the septic episodes that result from the patients' severe immunosuppression (2–4, 9). Depressed T-cell proliferation to mitogen and depressed IL-2 production have been hallmarks of trauma patients' immunosuppressed state (9–13). Recently, experiments using an *in vivo* murine system seem to suggest that excessive cytokine production in response to septic challenge could be reversed and mortality decreased by administration of the recently described cytokine IL-10 (14–16). In contradiction to these findings, other experiments in murine systems of hemorrhage-induced immunosuppression have reported increased splenocyte IL-10 production after injury and suggested excessive IL-10 production as responsible for postinjury immunosuppression in this murine system (17, 18).

IL-10 was originally described as a murine cytokine product of Th2 type clones that inhibited murine Th1 lymphokine production by decreasing monocyte (MØ) activation of Th1 cells (19, 20). Recent data demonstrated that monocytes/macrophage, B cells, $CD4^+$ Th1 clones, $CD8^+$ T cells, and mast cells can all produce various amounts of IL-10, which can then regulate a variety of lymphocyte and myeloid cell functions and suppress inflammatory cytokine production of T cells, monocytes/macrophage, and PMN (19–24). Although MØ appear to be the primary IL-10 source in stimulated normal human PBMC cultures, activated T cells may also be an important IL-10 source in trauma patients (21, 25–27). The data demonstrating that human IL-10 was a potent suppressor of human T-cell proliferation, both directly in a MØ independent system, and indirectly through its action on APC, suggest that IL-10 may be elevated after injury and responsible for posttrauma immunosuppression (19, 27–29). In contrast, the ability of IL-10 to down-regulate MØ, T cells, and PMN proinflammatory cytokines supports the hypothesis that IL-10 levels are inadequate after injury and that their

[1] Department of Surgery and the Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, Massachusetts.
[2] To whom correspondence should be addressed at Department of Surgery, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, Massachusetts 01655.

0271-9142/95/0300-0093$07.50/0

increase may be beneficial (14, 19, 22–25, 30). In the present study, we have examined PBMC, MØ, and T-cell IL-10 production by trauma patients who also are severely immunosuppressed and who may also have hyperelevated MØ TNF-α levels. IL-10 levels produced by these selected trauma patients' T lymphocytes and MØ are compared to IL-10 levels produced by trauma patients without severe immunosuppression and to paired normal controls.

MATERIALS AND METHODS

Patient Population. A total of 31 patients admitted to the University of Massachusetts Medical Center Trauma Unit, Worcester, was included in this study. Fifteen patients with mechanical trauma (injury severity score >30) and 16 patients with thermal trauma (>20% total-body surface burns) were assessed. Their ages ranged from 19 to 85; the median age was 35. There were 23 men and eight women. Normal controls were tested along with each patient. Volunteers from laboratory and hospital staff at the University of Massachusetts Medical Center (ages 20–58) served as normal controls. Informed consent was obtained from all patients and controls and the study was approved by the Institutional Review Board.

Cytokines. Recombinant human interleukin-10 (IL-10) was a generous gift from Schering-Plough Research Institute, Kenilworth, NJ. Recombinant human tumor necrosis factor alpha (TNF-α) was obtained from Collaborative Biomedical Products, Bedford, MA. Recombinant human transforming growth factor beta (TGF-β) was generously provided by Genentech, Inc., South San Francisco, CA, supplied in 20 m*M* NaAc, pH 5.0. Activity of TGF-β was confirmed in the sensitive mink lung (MvLu) bioassay.

Monocyte Separation and Stimulation. Monocytes from patients' and normals' blood were isolated from Ficoll-Hypaque gradient-separated mononuclear cells (PBMC) by selective adherence as described previously (31). Briefly, nonadherent cells were removed after 1.5 hr of adherence to microexudate-treated plastic surface, resulting in a >95% MØ purity as determined by fluorescein isothiocyanate (FITC) -labeled OKM5 staining. FcγRI cross-linked populations were obtained from normals by rosetting the MØ with human O, RhO(D)$^+$ erythrocytes (Selectogen, Ortho Diagnostic System, Inc., Raritan, NJ), and treated with anti-RhO(D) human immunoglobulin (RhO-GAM, Ortho Diagnostic System) as previously described (32, 33). This rosetting technique provides cross-linking stimulation of the high-density FcγRI-bearing (FcγRI$^+$) MØ subpopulations and yields an enriched FcγRI$^+$ population (33, 34). Three million MØ per well were cultured in 3.0 ml of RPMI 1640 medium (JRH Biosciences, Lenexa, KS), supplemented with 15% FBS (Sigma Chemical Co., St. Louis, MO), 50 units/ml penicillin-G, 50 μg/ml streptomycin, 50 μg/ml gentamicin, 2.5 μg/ml fungizone, 4 m*M* L-glutamine, 1 m*M* Na pyruvate, and 1% minimal essential medium (MEM) nonessential amino acids (JRH Biosciences). Endotoxin contamination was less than 15 pg/ml in the culture media and FBS, and all media contained 100 units/ml polymyxin B sulfate (GIBCO Laboratories, Grand Island, NY).

Normals' and patients' MØ were stimulated with 20 μg/ml MDP, a gram-positive cell wall analog (compound CGP 11637 was generously provided by CIBA-Geigy, Basel, Switzerland). Recombinant human IL-10 (50 units/ml) was added, along with MDP, in a number of experiments with normals' MØ. In some of the experiments with normals' MØ, 200 units/ml recombinant human TNF-α was added either alone or in combination with 20 μg/ml MDP. Similarly, 2.4 ng or 4.8 ng/ml TGF-β was added along with 20 μg/ml MDP in some experiments. MØ supernates were collected after 16–18 hr of stimulation and kept frozen at −80°C until the cytokine assays were performed. Adherent MØ were collected by EDTA treatment and scraping. Recovered cells were kept frozen at 5×10^6/ml concentration in PBS for further analysis.

Mitogen Assays. PBMC (2×10^5 cells/200 μl/well) were cultured in flat-bottomed microtiter plates (Becton Dickinson, Lincoln Park, NJ) in presence or absence of PHA (Murex Diagnostics Ltd., Dartford, England). The experiments were set up in duplicate. In one set, supernates were harvested after 30 hr and stored at −80°C until the day of assay for IL-10. In another set, cells were cultured for 72 hr for proliferation assays by [^{3}H]thymidine incorporation at the last 18 hr of incubation.

T-Cell Purification and Stimulation. PBMC T cells were purified from PBMC as previously described (32, 35). Briefly, normals' and patients' PBMC were depleted of MØ by selective adherence to microexudate-treated plastic surfaces. Nonadherent cells were rosetted with neuraminidase (Sigma) -treated sheep red blood cells (SRBC). The SRBC-rosetted cells were >90% T cells with <1% contamination by B cells or monocytes, as determined by flow cytometric analysis. The purified T cells were cultured (2×10^5 cells/200 μl/well) in flat-bottomed microtiter plates in presence of immobilized anti-CD3 and anti-CD4. MAb were immobilized onto plastic microtiter plates as described (36). In brief, anti-CD3 (Boehringer Mannheim, Indianapolis, IN), diluted in RPMI 1640 was placed (1.5 μg/50 μl/well) in

each of the wells of 96-well flat-bottomed microtiter plates (Becton Dickinson), incubated at room temperature for 1.5 hr, and then washed with PBS two times to remove nonadherent MAb. The process was repeated with anti-CD4 (Biosource International, Camarillo, CA) (1 μg/50 μl/well). After 24 hr of culture, 100 μl of supernates were harvested from each well and replenished with 100 μl of fresh medium and continued for proliferation for another 48 hr in a [^{3}H]thymidine incorporation assay.

TNFα Bioassay. TNF-α activity in MØ supernates (secreted TNF-α) and sonicated MØ lysates (cell-associated TNF-α) were measured in the L–M cell bioassay as previously described (33). Both cell-associated and secreted MØ TNF-α activity were totally inhibited by anti-TNF-α neutralizing antibody.

IL-10 ELISA. IL-10 levels in the supernates of PBMC, MØ, and T cells were determined by a specific ELISA kit (Biosource International) according to the protocol recommended by the manufacturer. The sensitivity of the assay was 5 pg/ml.

Statistical Analysis. Because of the known individual genetic variation in cytokine levels, parametric statistical analysis (i.e., mean and standard deviation) is inappropriate. Data for normals and patients in each assay are paired, but the individual samples analyzed in a Wilcoxon nonparametric assay (Macintosh Statview) treat each individual as coming from an independent population.

RESULTS

Examination of Trauma Patients' PBMC and MØ Supernates for IL-10 Levels. The depressed mitogen responses that typify burn and trauma patients have been suggested as resulting from increased PGE_2 levels, decreased IL-2 production, increased TGF-β levels, and/or depletion of T-lymphocyte numbers (7–12, 18, 37). IL-10 levels have also been suggested as being increased after severe hemorrhage and possibly mediating post-trauma immunosuppression (17, 18). Since IL-10 has been shown to depress costimulation of T-cell proliferation and to directly decrease T-lymphocyte IL-2 levels, it seemed reasonable that IL-10 levels might be increased in the PBMC supernates of trauma patients whose PHA mitogen-induced proliferation is depressed (19, 27–29). However, when the supernates from PHA-stimulated immunosuppressed patients' PBMC were assayed for IL-10 levels by ELISA and compared to simultaneously stimulated assayed normals' PBMC supernates, the levels of IL-10 were significantly (P = 0.002 Wilcoxon) depressed in the PHA-induced PBMC supernates of patients who were concomitantly experiencing decreased mitogen responses (Table I). The surprising finding that IL-10 levels were depressed in these immunosuppressed patients' PBMC led us to examine isolated MØ production of IL-10 in trauma patients who experienced depressed PHA responses. MØ are the primary producers of IL-10 in the freshly isolated and stimulated human PBMC population (25, 26). It was possible that excessive production of T lymphokines in the PBMC populations was decreasing the MØ IL-10 production or that an early *in vivo* stimulated increase of IL-10 in the patients' PBMC was autodepressing their subsequent *in vitro* MØ IL-10 production (22).

We examined the IL-10 production of isolated patients' and normals' MØ either cultured alone for 16 hr or stimulated 16 hr with the bacterial cell wall analog MDP (20 μg/ml). When patients' MØ IL-10 levels were assessed sequentially over time after injury, the IL-10 levels were seen initially to be in the normal range, then decrease dramatically at five to nine days after injury (Fig. 1). IL-10 levels would then begin to renormalize at later postinjury periods in recovering patients (Fig. 1). Consequently, individual patients' MØ IL-10 levels were repeatedly assessed at three to four day intervals over their clinical course and treated as independent samples. A particular patient's MØ IL-10 data and corresponding mitogen response might be normal, depressed, or elevated, depending on the postinjury day on which the sample was collected. As can be seen in Table II, those trauma patients whose PBMC responses were suppressed also exhibited significant reduction in their IL-10 levels either from MØ cultured alone or from MDP-stimulated MØ when compared to controls. In fact, when 37 samples of MØ supernates collected from 24 mitogen-depressed patients at different postinjury days were assayed for IL-10 levels, all patient samples showed a statistically significant (P = 0.0001 Wilcoxon) depression in both unstimulated and stimulated levels of MØ IL-10 as compared to their paired controls.

As illustrated by the six representative experiments in Fig. 2, there was significant variation in the assayed levels of IL-10 for the normals' MØ. However, in all 37 assays, the level of IL-10 produced by the patient MØ sample was depressed as compared to paired normal's. The median levels of IL-10 in cultured and MDP-stimulated normals' MØ samples were 0.910 and 2.553 ng/10^6 MØ/ml, respectively. The corresponding median IL-10 levels for the unstimulated and stimulated trauma patients' MØ were 0.128 and 0.461 ng/10^6 MØ/ml, showing significant suppression (P = 0.0001) when the entire group was analyzed with the Wilcoxon nonparametric test. We also considered the possibility

Table I. Depressed IL-10 Production by Immunosuppressed Trauma Patients' PBMC

Experiment	% decrease proliferation[a]	IL-10 (ng/10^6 PBMC/ml)[b] Normal	Patient[c]
1	41	5.350	1.160
2	65	4.005	1.850
3	81	2.850	0.320
4	42	3.600	<0.005
5	96	2.040	0.460
6	66	4.520	0.380
7	57	4.080	0.930
8	98	4.600	<0.005
9	85	5.100	0.360
10	71	2.900	1.470
11	71	4.600	0.790
12	67	1.640	0.340
		$P = 0.0022$[d]	

[a] PHA-induced proliferation calculated from [^{3}H]thymidine incorporation using the formula $A - B/A \times 100$ where A = counts for normal, B = counts for patient; and the patients having >30% decrease in proliferation were considered immunosuppressed.

[b] PBMC were cultured in 96-well plates (2×10^5 cells/200 μl/well) in the presence of PHA. After 24 hr of culture, supernates were harvested and assessed for IL-10 by ELISA.

[c] Twelve samples were collected from six patients at different postinjury days.

[d] Statistical significance (P) between normal and patient values determined by Wilcoxon nonparametric test.

that early elevated MØ IL-10 levels stimulated *in vivo* by the trauma might be suppressing subsequent IL-10 production *in vitro*, since IL-10 is autosuppressive (22). We examined three patients' and paired normals' MØ IL-10 levels at both 3 h and 16 hr after culture. The 3-hr MØ IL-10 levels of the patients were unmeasurable, as were the normals' IL-10 levels. After 16 hr, the normals' MØ produced measurable IL-10 levels, while the patients' MØ IL-10 levels were significantly decreased (data not shown). These data suggest that early *in vitro* MØ production of IL-10 is not responsible for decreasing IL-10 levels at the time we assay. In addition, the data show that MØ-produced IL-10 is not responsible for the decreased mitogen-induced proliferation of these trauma patients since IL-10 levels are depressed in these patients.

Examination of T-Cell IL-10 in Immunosuppressed Patients and Normals. MØ are the primary IL-10 source in stimulated human PBMC (19, 25, 26). Consequently, the decreased MØ IL-10 levels in the PHA-stimulated supernates from patients' PBMC might mask decreased or increased IL-10 production by the patients' T cells. Since T-cell numbers are depleted after injury, the assay of PBMC supernates might fail to detect altered IL-10 levels in the remaining T-cell population. In view of reports of increased IL-10 production in murine $CD4^+$ splenocytes posthemorrhage when the whole splenocyte population showed no change, the patients' isolated T lymphocytes also needed to be examined for IL-10 levels (17). We have previously shown that patients with depressed PBMC mitogen responses could display either

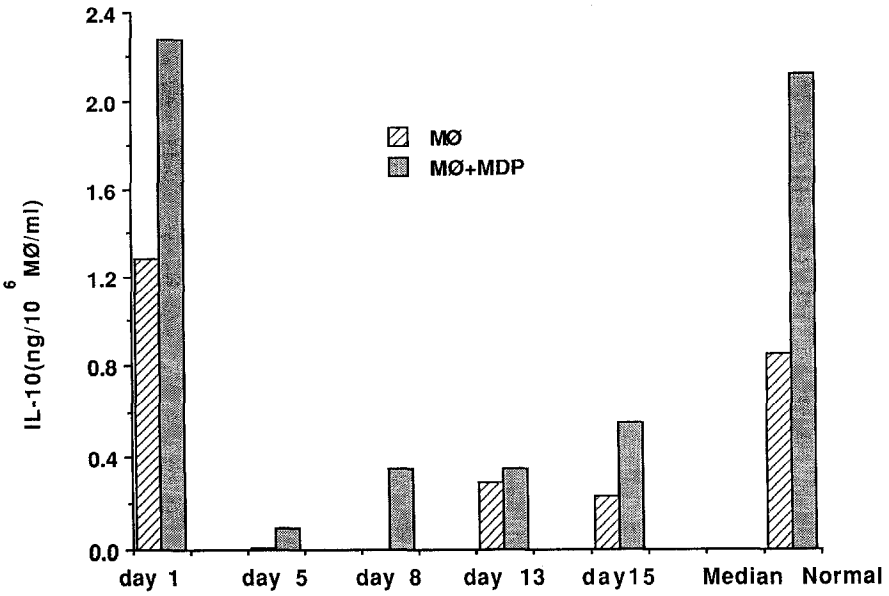

Fig. 1. MØ IL-10 levels of one patient at five different postinjury days. MØ from the patient and control normal were cultured (3×10^6 cells/3 ml) for 16 h in medium alone (MØ) or in the presence of MDP (20 μg/ml), and IL-10 levels in the culture supernates were measured by ELISA. Median values of MØ IL-10 levels of five control normals are included in the figure.

Table II. Depressed MØ IL-10 Production in Immunosuppressed Trauma Patients

		MØ IL-10 (ng/10^6-MØ/ml)[b]			
		Unstimulated[c]		MDP[e]	
Experiment	% decrease proliferation[a]	Normal	Patient[d]	Normal	Patient
1	39	2.775	0.031	4.687	0.075
2	91	3.186	<0.005	4.329	<0.005
3	73	0.621	0.083	2.451	0.134
4	67	0.265	<0.005	1.139	0.033
5	70	0.325	<0.005	1.022	<0.005
6	70	0.942	0.148	2.809	0.230
7	42	2.081	<0.005	3.024	0.099
8	51	0.480	0.013	1.313	0.096
9	72	0.623	<0.005	2.053	0.357
10	65	0.508	0.089	1.934	0.604
11	80	3.781	<0.005	10.466	0.461
12	55	3.559	0.686	6.195	0.700
13	79	0.860	<0.005	2.216	0.493
14	42	3.107	0.149	6.964	0.704
15	98	2.515	<0.005	5.944	<0.005
16	71	2.515	0.268	5.944	0.755
		$P = 0.0004$[f]		$P = 0.0004$[f]	

[a] PHA-induced proliferation calculated from [^{3}H]thymidine incorporation using the formula $A - B/A \times 100$ where A = counts for normal, B = counts for patient; and the patients having >30% decrease in proliferation were considered immunosuppressed.
[b] IL-10 levels in the MØ culture supernates measured by ELISA.
[c] MØ (3×10^6 cells/3 ml) cultured in medium alone for 16 hr.
[d] Sixteen samples collected from 12 patients at different postinjury days.
[e] MØ (3×10^6 cells/3 ml) cultured in medium and MDP (20 μg/ml) for 16 hr.
[f] Statistical significance (P) between normal and patient values determined by Wilcoxon nonparametric test.

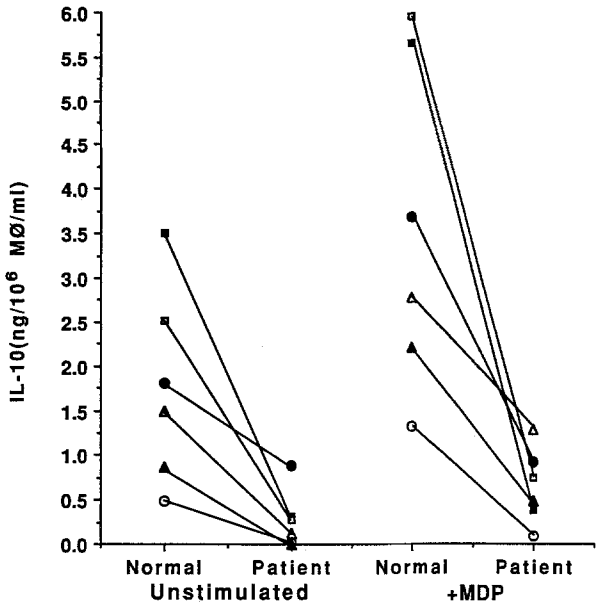

Fig. 2. Data of six representative experiments from 37 experiments, showing depressed MØ IL-10 production in patients (P value for normal unstimulated vs patient unstimulated = 0.0001 and for normal MDP vs patient MDP = 0.0001 for all the experiments, as assessed by Wilcoxon nonparametric test). MØ from both normals and trauma patients were cultured (3×10^6 cells/3 ml) for 16 hr in medium alone (unstimulated) or in the presence of MDP (20 μg/ml) and the IL-10 levels in the culture supernates were measured by ELISA.

depressed or normal T-cell proliferation in a MØ-independent T-cell proliferation system (38). We have also demonstrated that many immunocompetent trauma patients have highly elevated mitogen proliferation, and these patients also were found to have elevated T-cell proliferation (13). The trauma patients were, therefore, divided into three groups based on their altered mitogen and T cell proliferative responses. Patients with unaltered mitogen responses were not included. The groups are: patients with depressed mitogen responses and depressed T-cell proliferation, patients with depressed mitogen responses and normal or slightly elevated T-cell proliferation, and patients with elevated PHA and elevated T-cell proliferation. Patient and paired normal purified T cells from these three groups were assessed for IL-10 levels after stimulation by immobilized anti-CD3 and anti-CD4 (36, 38).

IL-10 has been previously shown to inhibit anti-CD3-stimulated human T-cell proliferation (28). Consequently, any suppressive effect of increased IL-10 produced by patients' T cells should be detectable in this MØ-independent T-cell proliferation assay. IL-10 levels were much lower in this MØ-independent T-cell proliferation system than in either PHA-stimulated PBMC or in MDP-stimulated isolated MØ. Nevertheless, as can be seen in Table III, patients with depressed mitogen responses and depressed T-cell proliferation in this T cell assay also had significantly depressed IL-10 production (Wilcoxon $P = 0.007$) as compared to the paired normals. These data indicate that increased T-cell production of IL-10 was not mediating decreases in the T-cell proliferation evidenced by these patients. All but one of the patients with depressed T-cell responses and depressed IL-10 levels went on to die. In striking contradiction, when we examined the T-cell levels of IL-10 produced by trauma patients with depressed mitogen responses, but normal or elevated T-cell proliferation, we found significantly elevated IL-10 production (Wilcoxon $P = 0.018$) as compared to the normals (Table IV). Elevated mitogen responses in trauma patients' PBMC have been previously attributed to a normal immune response to an ongoing bacterial and/or other *in vivo* stimulation (13). Patients with elevated mitogen responses had massively elevated T-cell proliferation and very elevated IL-10 levels (Table V). This IL-10 elevation in some immunocompetent trauma pa-

Table III. Depressed T Cell IL-10 Production in Trauma Patients with Depressed T Cell Proliferation[a]

Experiment	% decrease T cell proliferation	IL-10 (pg/10^6 T cells/ml)[b] Normal	Patient[c]
1	99	39	<5
2	97	117	<5
3	83	117	37
4	94	64	<5
5	60	108	<5
6	31	100	37
7	32	53	<5
8	56	156	<5
9	54	63	<5
		$P = 0.007$[d]	

[a] T cell proliferation was assessed in a [^{3}H]thymidine incorporation assay by culture of monocyte-depleted SRBC-rosetted T cells (2×10^5 cells/200 μl/well) in presence of immobilized anti-CD3 (1.5 μg/well) plus anti-CD4 (1 μg/well) for 72 hr. When the proliferation of T cells from the patient is decreased >30% as compared to the paired normal, it is considered depressed.

[b] IL-10 levels in the supernates of T cells cultured in presence of immobilized anti-CD3 (1.5 μg/well) plus anti-CD4 (1 μg/well) for 24 hr, were measured by ELISA.

[c] Nine samples were collected from four patients at different postinjury days.

[d] Statistical significance (*P*) between normal and patient values was determined by Wilcoxon nonparametric test.

Table IV. T Cell IL-10 Production in Trauma Patients with Normal or Elevated T Cell Proliferation[a]

Experiment	T cell proliferation (dpm × 10^{-3}) Normal	Patient	IL-10 (pg/10^6 T cells/ml)[b] Normal	Patient[c]
1	17	13	117	162
2	14	24	34	135
3	9	20	10	20
4	19	12	5	14
5	53	70	62	744
6	45	47	156	468
7	19	19	63	93
8	22	26	46	46
			$P = 0.018$[d]	

[a] T cell proliferation was assessed in a [^{3}H]thymidine incorporation assay by culture of monocyte-depleted SRBC-rosetted T cells (2×10^5 cells/200 μl/well) in presence of immobilized anti-CD3 (1.5 μg/well) plus anti-CD4 (1 μg/well) for 72 hr and expressed as dpm.

[b] IL-10 levels in the supernates of T cells cultured in presence of immobilized anti-CD3 (1.5 μg/well) plus anti-CD4 (1 μg/well) for 24 hr, were measured by ELISA.

[c] Eight samples were collected from five patients at different post injury days.

[d] Statistical significance (*P*) between normal and patient values was determined by Wilcoxon nonparametric test.

tients might reflect normally increased T-cell activity after *in vivo* stimulation. Even though the patients' PBMC mitogen response may be depressed, if their isolated T-cell proliferation is normal or slightly elevated, then their IL-10 levels can be elevated. These data indicate that trauma patients' T cells have depressed, not elevated, IL-10 production when their T-cell proliferation is compromised, but can have elevated IL-10 production if their T cell proliferation is normal or elevated. In contrast, patients' MØ IL-10 production is always depressed when their PBMC mitogen responses are depressed.

Relationship of Elevated Patients' MØ TNF-α to IL-10 Levels. If MØ IL-10 levels are depressed after trauma in the face of the massive postinjury MØ stimulation by trauma-generated fibrin degradation products, complement split products, and bacterial stimuli, it could have profound effects on posttrauma production of monokines. MØ-produced IL-10 is suggested as playing a major down-regulatory role in normals' MØ TNF-α production and as contributing to the sharp peak and decline of TNF-α production after MØ stimulation (19, 22). TNF-α, IL-6, and PGE_2 are all known to be excessively elevated after trauma and to persist over a prolonged period after injury (2–7). We initially thought that the elevated TNF-α levels occurring after trauma might be suppressive to MØ production of IL-10. However, when we examined the effect of exogenous TNF-α addition to normal MØ, we found that IL-10 levels were actually increased by addition of TNF-α to the culture media (Fig. 3). Similar data were recently published showing TNF-α augmentation of normals' MØ IL-10 production (39). Since immunosuppressed trauma patients' MØ produced elevated TNF-α levels in response to MDP stimulation, we questioned what the IL-10 levels would be in MØ supernates from such patients. As illustrated in Table VI, those patients' MØ with hyperincreased TNF-α responses to MDP had simultaneously depressed IL-10 responses as compared to paired normals'. Although the level of TNF-α still detectable at 16 hr of culture with MDP is variable with different normals' MØ, their IL-10 levels are always stimulated. In contrast, those patients' MØ that show persistently high TNF-α levels after 16 hr of MDP stimulation show significantly depressed IL-10 levels. These data suggest that patients' MØ with exaggerated TNF-α responses may also fail to produce IL-10 and consequently fail to rapidly down-regulate their TNF-α levels after activation.

Sensitivity of Patients' MØ TNF-α to IL-10 Down-Regulation

A recent report suggested that plasma IL-10 levels were elevated, not depressed, in patients with septic shock (40). Although these septic patients were not

Table V. Increased Mitogen Induction and T-Cell Proliferation Concomitant to Increased IL-10

Experiment	% increase PHA response[a]	T cell proliferation (dpm × 10^{-3})[b] Normal	Patient[c]	IL-10 (pg/10^6 T cells/ml)[d] Normal	Patient
1	485	49	104	122	175
2	100	17	47	8	94
3	89	9	22	10	59
4	187	19	25	88	660
5	65	11	23	20	41
6	40	10	56	26	76
7	80	22	150	9	175
8	89	18	65	8	48
		$P = 0.0117$[e]		$P = 0.0117$[e]	

[a] PHA-induced proliferation calculated from [^{3}H]thymidine incorporation using the formula $B - A/A$ X 100 where A = baseline proliferation of patient's PBMC measured by day 1 after injury and B = proliferation of PBMC of the same patient corresponding to the day of assessment of T-cell proliferation and IL-10 production.

[b] T-cell proliferation was assessed in a [^{3}H]thymidine incorporation assay by culture of monocyte-depleted SRBC-rosetted T cells (2×10^5 cells/200 μl/well) in presence of immobilized anti-CD3 (1.5 μg/well) plus anti-CD4 (1 μg/well) for 72 hr and expressed as dpm.

[c] Eight samples were collected from three patients at different postinjury days.

[d] IL-10 levels in the supernates of T cells cultured in presence of immobilized anti-CD3 (1.5 μg/well) plus anti-CD4 (1 μg/well) for 24 hr, were measured by ELISA.

[e] Statistical significance (P) between normal and patient values was determined by Wilcoxon nonparametric test.

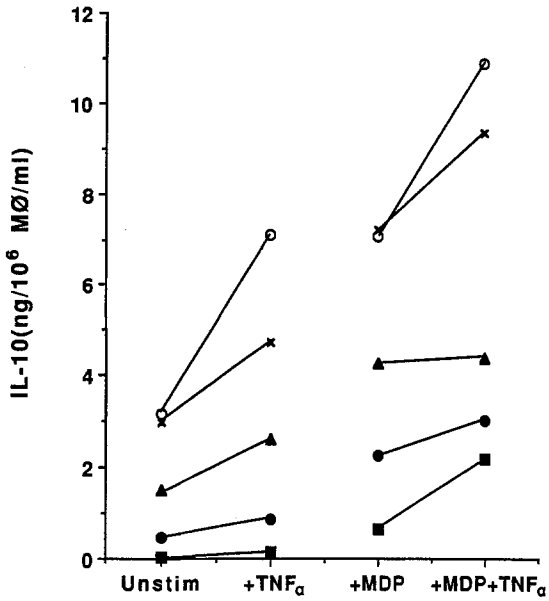

Fig. 3. Data of five representative experiments from 10 experiments, showing induction of MØ IL-10 production of TNF-α (P value for unstimulated vs TNF-α-stimulated = 0.018 and for MDP vs MDP + TNF-α = 0.0117 for all the experiments, as assessed by Wilcoxon nonparametric test). MØ from normals were cultured (3×10^6 cells/3 ml) for 16 hr in medium alone (unstimulated), MDP (20 μg/ml), TNF-α (200 units/ml), or MDP (20 μg/ml) in presence of TNF-α (200 units/ml) and the IL-10 levels in the culture supernates were measured by ELISA.

immunosuppressed trauma patients, these data suggested that elevated MØ TNF-α production might be persisting in the presence of high IL-10 production after trauma. MØ TNF-α production after trauma, has been described as resistant to down-regulation by PGE_2 (7, 8). In addition, IL-10 down-regulation of monokines has also been reported to vary depending on the inducing stimuli (41). Consequently, trauma patients' MØ production of TNF-α in response to nonbacterial stimuli might be insensitive to IL-10 down-regulation, thereby providing an additional explanation for elevated MØ TNF-α production after injury. Exogenous recombinant human IL-10 was added to cultures of patients' and normals' MØ at 50 units/ml. We have previously shown that this IL-10 concentration suppresses >95% of the normal MØ levels of TNF-α produced in response to MDP stimulation (data not shown). In this set of experiments, paired normals' and immunosuppressed trauma patients' MØ were assessed for MDP-induced TNF-α production in the presence and absence of IL-10. As illustrated in Table VII, the elevated levels of TNF-α produced by the patients' MØ were still sensitive to the down-regulatory effects of IL-10, although in several cases significant TNF-α was still produced by patients' MØ in the presence of IL-10 addition. However, these patients' MØ had elevated TNF-α levels before MDP stimulation (data not shown). Since IL-10 can suppress TNF-α mRNA at both the transcriptional and translational levels, these data suggest that trauma patients' MØ *de novo* production of TNF-α in response to continued stimuli is still sensitive to IL-10 down-regulation (22, 42).

Effect of TGF-β on MØ IL-10 Production. In addition to TNF-α, trauma patients' MØ produce highly elevated levels of TGF-β (37). TGF-β is a potent down-regulator of a number of cytokines (35, 42). In a small series of experiments, we examined the effects of exogenous TGF-β on MØ IL-10 levels. As illustrated by five representative experiments of 13 (Fig. 4), addition of 2.4 ng/ml recombinant human TGF-β_1 to normal MØ cultures significantly depressed their IL-10 production (Wilcoxon $P = 0.0003$). Although these data indicate that TGF-β can depress normal MØ IL-10 levels, this mechanism may not be operative in the trauma patients where a variety of MØ stimuli are present in addition to bacterial challenge. We have previously presented data indicating that trauma patients' monokine production is stimulated by *in vivo* cross-linking of MØ FcγRI receptors by the massively increased IgG levels in these patients' circulation (43, 44). In addition, we and others

Table VI. Trauma Patients with Elevated MØ TNF-α Levels also Have Depressed MØ IL-10 Levels

Experiment	MØ TNF-α (ng/10^6 MØ/ml)[a]		MDP-induced MØ IL-10 (ng/10^6 MØ/ml)[c]	
	Normal	Patient[b]	Normal	Patient
1	0.847	5.755	2.094	0.728
2	1.154	11.603	5.660	0.393
3	<0.05	7.262	1.139	<0.005
4	8.342	68.875	2.842	0.348
5	8.103	28.586	2.555	<0.005
6	<0.05	4.813	10.466	0.461
7	4.915	10.167	2.216	0.493
8	6.125	12.372	6.964	0.704
9	<0.05	24.224	5.944	0.755
	$P = 0.0077$[d]		$P = 0.0077$[d]	

[a] TNF-α levels, cell-associated (MØ lysate) plus secreted (MØ supernates), of MØ cultured in medium and MDP (20 μg/ml) for 16 hr, were assessed by LM bioassays.
[b] Nine samples were collected from seven patients at different post injury days.
[c] IL-10 levels in the supernates of MØ cultured in medium and MDP (20 μg/ml) for 16 hr, were measured by ELISA.
[d] Statistical significance (*P*) between normal and patient values determined by Wilcoxon nonparametric test.

Table VII. IL-10 Down-Regulates MØ TNF-α Production in Normals and Patients

Experiment	MØ TNF-α (ng/10^6 MØ/ml)[a]			
	Normal		Patient[d]	
	MDP[b]	MDP + IL-10[c]	MDP	MDP + IL-10
1	5.65	<0.05	17.64	12.17
2	5.65	<0.05	20.2	5.4
3	2.37	<0.05	7.53	4.34
4	1.34	<0.05	35.6	4.23
5	12.36	6.25	22.57	16.90
6	1.26	<0.05	26.0	<0.05
7	3.97	0.78	8.33	1.05
8	4.82	1.43	11.13	2.86
9	2.97	<0.05	5.54	<0.05
	$P = 0.0076$[e]		$P = 0.0077$[e]	

[a] TNF-α levels, cell associated (MØ lysate) plus secreted (MØ supernates), were assessed by LM bioassays.
[b] MØ (3×10^6 cells/ml) were cultured in medium and MDP (20 μg/ml) for 16 hr.
[c] MØ (3×10^6 cells/3 ml) were cultured in medium, MDP (20 μg/ml) and IL-10 (50 units/ml) for 16 hr.
[d] Nine samples were collected from four patients at different postinjury days.
[e] Statistical significance (*P*) between MDP vs MDP + IL-10-stimulated TNF-α values was determined by Wilcoxon nonparametric test.

have shown that FcγRI cross-linking induction of normals' MØ induces cytokine responses, some of which parallel those observed by trauma patients' MØ (33). Consequently, we asked if IL-10 induced by FcγRI cross-linking stimulation of MØ followed by MDP stimulation could also be down-regulated by exogenous TGF-β addition, thereby testing the TGF-β down-regulatory effect on IL-10 in a more intensely stimulated MØ. Addition of 4.8 ng/ml of TGF-β would decrease the IL-10 induced by MDP stimulation subsequent to FcγRI cross-linking, as can be seen in the five representative experiments in Fig. 5. These data imply that patients' MØ IL-10 production could be suppressed by concomitant induction of TGF-β. Although trauma patients' MØ with elevated TGF-β levels always exhibited depressed IL-10 induction, some patients' MØ had depressed IL-10 induction capacity but no elevated TGF-β levels (data not shown). These data suggest that although postinjury hyperelevation of TGF-β levels can contribute to depressed MØ IL-10 levels in the posttrauma patient, they cannot be the sole explanation for the decreased MØ IL-10 production in immunosuppressed trauma patients.

DISCUSSION

Although there are no reports on posttrauma IL-10 levels, two recent reports suggesting that IL-10 levels were increased after hemorrhage, and another report of increased plasma levels of IL-10 in septic patients, would seem to imply that elevated IL-10 levels could be mediating the severe immunosuppression occurring in trauma patients (17, 18, 40). These data are difficult to reconcile with the highly elevated TNF-α and IL-6 production that is simultaneously occurring in many of these immunosuppressed patients and is directly associated with the posttrauma organ failure and mortality that occurs one to three weeks after the injury (2–7). IL-10 has been shown to suppress both MØ and PMN production of inflammatory cytokines (22–25). The data reporting elevated IL-10 after sepsis is also in contrast to a number of *in vivo* reports showing that animals depleted of IL-10 have dramatically increased susceptibility to endotoxin shock mortality and that administration of IL-10 can significantly improve survival during endotoxin shock by depressing TNF-α levels (14–16, 45). Other murine studies have shown that IL-10 protects from lung injury and inhibits MØ procoagulant activity, thereby decreasing lung failure due to disseminated intravascular coagulation (DIC) (46, 47). DIC is a common posttrauma sequel and would not be expected to occur concomitant with elevated IL-10 levels, again suggesting that IL-10 levels should be suppressed after trauma.

The data reported here may reconcile some of these seemingly disparate reports. Our trauma patients were separated into three groups based on their T-cell proliferative responses in comparison to normal controls. One group had both depressed mitogen responses in the

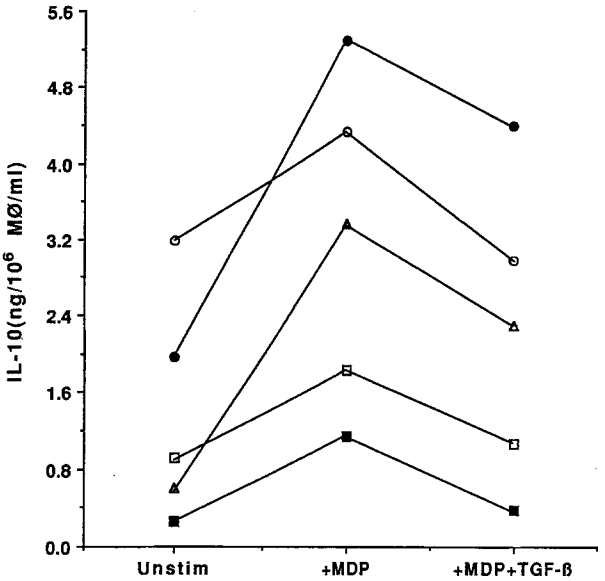

Fig. 4. Data of five representative experiments from 13 experiments, showing down-regulation of MDP induced MØ IL-10 production by TGF-β (*P* value for MDP vs MDP + TGF-β = 0.0003 for all experiments, as assessed by Wilcoxon nonparametric test). MØ from normals were cultured (3×10^6 cells/3 ml) for 16 hr in medium alone (unstimulated), MDP (20 μg/ml), or MDP (20 μg/ml) in presence of TGF-β (2.4 ng/ml) and the IL-10 levels in the culture supernates were measured by ELISA.

PBMC population and depressed T-cell proliferation in a MØ-independent proliferation system (anti-CD3 and anti-CD4 stimulation). The second patient group had depressed mitogen responses in the PBMC population, but normal or slightly elevated T-cell proliferation. The third patient group had elevated mitogen responses and significantly elevated T-cell proliferation. The IL-10 levels of T cells from these three groups of patients were dramatically different, while MØ IL-10 levels in both the mitogen-suppressed groups were depressed. Patients with depressed mitogen responses and depressed T-lymphocyte proliferation had both significantly depressed T-cell and MØ IL-10. In contrast, patients with depressed mitogen responses but normal or elevated T-cell proliferation capacity produced normal or elevated levels of T-cell IL-10 in response to anti-CD3, anti-CD4 stimulation, but had depressed MØ IL-10 levels. Patients with elevated mitogen responses had both highly elevated T-cell proliferation and highly elevated T cell IL-10 levels. MØ from patients with normal PBMC proliferation in response to mitogen also had normal IL-10 production.

The posttrauma appearance of a depressed PBMC mitogen response in the face of competent T-lymphocyte proliferation is explainable by suppressed T-cell induction to PHA in the PBMC population, secondary to increased MØ PGE_2 and MØ TGF-β production, as well as reduced T-cell numbers in the PBMC population (6–8, 37). Since MØ are the primary producers of IL-10 in the mitogen-stimulated PBMC population, depressed MØ IL-10 production makes these patients' PBMC IL-10 levels appear depressed when compared to normals' (25, 26). However, these patients had elevated T-cell IL-10 responses induced by anti-CD3, anti-CD4 if their T-cell proliferation was normal or elevated. It has been demonstrated that exogenous IL-10 can directly inhibit human T-cell APC-independent proliferation in response to anti-CD3 stimulation (28). Consequently, the elevated IL-10 levels of our patients' T cells could not have been mediating their depressed PBMC mitogen responses since their APC-independent T-cell proliferation was still normal or elevated and their PBMC IL-10 levels were depressed.

The reported data showing elevated plasma levels of IL-10 in septic patients is compatible with our data showing highly elevated T-cell IL-10 levels in many trauma patients with normal or elevated T-cell proliferation responses. Since many trauma patients exhibit an elevated level of T-cell proliferation (possibly in response to infectious challenge), a detection of elevated plasma IL-10 might be explained. However, trauma

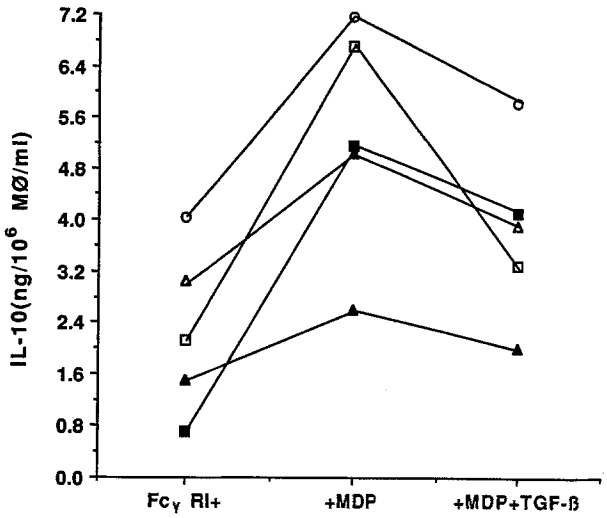

Fig. 5. Data of five representative experiments from 17 experiments, showing down-regulation of FcγRI cross-linked MDP-induced MØ IL-10 production by TGF-β (P value for MDP vs MDP + TGF-β = 0.0006 for all experiments, as assessed by Wilcoxon nonparametric test). MØ from normals, separated by rosetting with anti-Rh coated erythrocytes were cultured (3×10^6 cells/3 ml) for 16 hr in medium alone ($Fc\gamma RI^+$), MDP (20 μg/ml), or MDP (20 μg/ml) in presence of TGFβ (4.8 ng/ml), and the IL-10 levels in the culture supernates were measured by ELISA.

patients with elevated IL-10 resolved their infection and appeared to have normal immune responses. It was the trauma patients showing significantly decreased T-cell proliferation and depressed MØ and T-cell IL-10 production who went on to die, again suggesting that elevated IL-10 is not the mediator of trauma patients' immunosuppression. The data showing increased IL-10 production after hemorrhage in the murine systems may reflect known species differences, time of measurement differences, or a difference in hemorrhage alone versus multiple trauma. In one murine experiment, hemorrhage led to a three-day posthemorrhage increase in IL-10 production by isolated murine splenic $CD4^+$ T cells, *in vitro* stimulated with Con A, but no increase in IL-10 production by the whole posthemorrhage splenocyte population (17). Since there was no measurable IL-10 production in the total splenocyte population of either control or hemorrhage animals, elevated IL-10 levels could not have been affecting the total splenocyte proliferation responses. These hemorrhage model data showed increased IL-10 only in the isolated $CD4^+$ splenic cells.

Our data also include patients whose isolated T-cell IL-10 responses were increased when their total IL-10 levels in stimulated PBMC were depressed as compared to normals, but these patients had normal or elevated T-cell proliferation. Many of our patients initially appear to have had normal IL-10 levels, followed by a period of increased T-cell proliferation and increased T-cell IL-10 production, which then either progressed to severe immune suppression and decreased IL-10 levels or returned to a normal proliferation profile. The data are particularly intriguing because they may indicate an early selective increase in T-cell IL-10 production capacity detected by *in vitro* stimulation of the $CD4^+$ population. Patients with severe trauma show increasing T-cell depletion over time after injury (10). This depletion appears to result from T-cell apoptosis (48, 49). It might, therefore, be suggested that Th1 lymphocytes from trauma patients are more sensitive to posttrauma apoptosis as are Th1 lymphocytes from HIV patients (50). This would result in an initial increase in the proportion of Th2 lymphocytes that could result in the isolated $CD4^+$ population producing increased IL-10 while maintaining a normal or even elevated response to *in vitro* stimulation by anti-CD3, anti-CD4. However, such a Th1 depletion would need to be quickly followed by development of a total T-cell dysfunction with no T-cell proliferation and no IL-10 production to accommodate our immunosuppressed trauma patient data.

The early posttrauma loss of MØ IL-10 production could reflect such an early transient increase in T-cell IL-10, which then depresses MØ IL-10 levels. IL-10 depression of further MØ IL-10 production has been previously described (22). Excessive MØ TGF-β production could then also contribute to maintaining MØ IL-10 depression since elevated MØ TGF-β usually arises later in the postinjury period (37). During such an initial postinjury period of increased IL-10 production, inflammatory cytokine production would be appropriately controlled. If the T-cell dysfunction intensifies to include both Th1 and Th2 lymphocytes, as indicated by a failure of anti-CD3 and anti-CD4 to induce proliferation, then IL-10 levels are totally depressed and MØ and PMN production of inflammatory cytokines, such as TNF-α and IL-6, can proceed unabated. This model would incorporate our data showing depressed MØ and T-cell IL-10 production concomitant to increased MØ TNF-α levels, the *in vivo* murine data illustrating the protective effect of IL-10 against cytokine shock, and the recent murine hemorrhage model reports of early increased IL-10 levels. The hyperelevated MØ TNF-α levels seen in trauma patients more than seven days after injury were occurring concomitant with MØ inability to be induced for IL-10 production. Our data suggest that the severe posttrauma immunosuppression that occurs concomitant with hyperelevated cytokine levels is characterized by a loss of regulatory IL-10 production by both the MØ and T-cell populations. This loss of IL-10 activity may contribute to the overproduction of inflammatory cytokines, which is presumed to lead to patient mortality. In addition, IL-10 is important to B-cell maturation and has been reported to protect T cells from apoptosis (51, 52). Loss of IL-10 activity may, therefore, also allow increased T-cell apoptosis in the chronically stimulated T cells of trauma patients. A very early postinjury elevation in T-cell IL-10 levels may, therefore, represent a normal and appropriate response designed to temper the trauma-mediated activation of MØ, T cells, PMN, and B cells and prevent T-cell depletion. It is apparent, however, from the data presented here, that the severe T-cell immunosuppression that is associated with increased posttrauma mortality is not a result of elevated T-cell or MØ IL-10 production. Our data also suggest that the loss of MØ IL-10 production may be detrimental to the patients' regulation of inflammatory cytokines and may contribute to increased occurrences of end organ failure.

ACKNOWLEDGMENTS

This work was supported by the Public Health Service grant GM36214-09 and Department of Defense grant DAMD17-92-C-2033.

We appreciate the generous support of our clinical collaborators, Gary Fudem, MD, Lena Napolitano, MD, and Juan Carlos Puyana, MD, in identifying these patients. We would also like to thank the nurses of the Burn Unit and the Surgical I.C.U. for their help and support.

Human rIL-10 was a generous gift of Schering-Plough Research Institute, Kenilworth, NJ; transforming growth factor β was generously given by Genentech, Inc., South San Francisco, CA. We also appreciate the generous gift of the muramyl dipeptide from CIBA-Geigy, Basel, Switzerland.

REFERENCES

1. Waydhas C, Nast-Kolb D, Jochum M, Trupka A, Lenk S, Fritz H, Duswald K-H, Schweiberer L: Inflammatory mediators, infection, sepsis, and multiple organ failure after severe trauma. Arch Surg 127:460–467, 1992
2. Waage A, Aasen AO: Different role of cytokine mediators in septic shock related to meningococcal disease and surgery/polytrauma. Immunol Rev 127:221–230, 1992
3. Bitterman H, Kinarty A, Lazarovich H, Lahat N: Acute release of cytokines is proportional to tissue injury induced by surgical trauma and shock in rats. J Clin Immunol 11:184–192, 1991
4. Cavaillon J-M, Munoz C, Fitting C, Misset B, Carlet J: Trends in shock research. Circulating cytokines: The tip of the iceberg? Circ Shock 38:145–152, 1992
5. Suter PM, Suter S, Girardin E, Roux-Lombard P, Grau GE, Dayer J-M: High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, and elastase, in patients with adult respiratory distress syndrome after trauma, shock, or sepsis. Am Rev Respir Dis 145:1016–1022, 1992
6. Takayama T, Miller-C, Szabo G: Elevated tumor necrosis factor (TNF) production concomitant to elevated prostaglandin E_2 production by trauma patients' monocytes. Arch Surg 123:29–35, 1990
7. Molloy RG, O'Riordain M, Holzheimer R, Nestor M, Collins K, Mannick JA, Rodrick ML: Mechanism of increased tumor necrosis factor production after thermal injury. Altered sensitivity to PGE_2 and immunomodulation with indomethacin. J Immunol 151:2142–2149, 1993
8. Miller-Graziano CL, Kodys K, Jhaver K: MØ cell-associated TNFα is resistant to PGE_2 downregulation. Circ Shock Suppl 2:68, 1993
9. Guillou PJ: Biological variation in the development of sepsis after surgery or trauma. Lancet 342:217–220, 1993
10. Markewitz A, Faist E, Niesel Th, Lang S, Weinhold Ch, Reichart B: Changes in lymphocyte subsets and mitogen responsiveness following open-heart surgery and possible therapeutic approaches. Thorac Cardiovasc Surg 40:14–18, 1992
11. Gadd MA, Hansbrough JF: Postburn suppression of murine lymphocyte and neutrophil functions is not reversed by prostaglandin blockade. J Surg Res 48:84–90, 1990
12. Teodorczyk-Injeyan JA, Sparkes BG, Lalani S, Peters WJ, Mills GB: IL-2 regulation of soluble IL-2 receptor levels following thermal injury. Clin Exp Immunol 90:36–42, 1992
13. Miller CL, Baker CC: Changes in lymphocyte activity after thermal injury. The role of suppressor cells. J Clin Invest 63:202–210, 1979
14. Gerard C, Bruyns C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux A, Fiers W, Goldman M, Velu T: Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. J Exp Med 177:547–550, 1993
15. Howard M, Muchamuel T, Andrade S, Menon S: Interleukin 10 protects mice from lethal endotoxemia. J Exp Med 177:1205–1208, 1993
16. Bean A, Freiberg R, Andrade S, Menon S, Zlotnik A: IL-10 and/or IL-4 protect mice against staphylococcal enterotoxin B (SEB) induced lethal shock. J Immunol 150:8, 1993
17. Abraham E, Chang Y-H: Haemorrhage-induced alterations in function and cytokine production of T cells and T cell subpopulations. Clin Exp Immunol 90:497–502, 1992
18. Ayala A, Lehman DL, Herdon CD, Chaudry IH: Interleukin (IL)-10 induced suppression of T-cell responses following hemorrhage (HEM) is mediated by eicosanoids. Intensive Care Med 20:S59, 1994
19. Moore KW, O'Garra A, De Waal Malefyt R, Vieira P, Mosmann TR: Interleukin-10. Annu Rev Immunol 11:165–190, 1993
20. Spits H, De Waal Malefyt R: Functional characterization of human IL-10. Int Arch Allergy Immunol 99:8–15, 1992
21. Yssel H, De Waal Malefyt R, Roncarolo M-G, Abrams JS, Lahesmaa R, Spits H, De Vries JE: IL-10 is produced by subsets of human $CD4^+$ T cell clones and peripheral blood T cells. J Immunol 149:2378–2384, 1992
22. De Waal Malefyt R, Abrams J, Bennett B, Figdor CG, De Vries JE: Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. J Exp Med 174:1209–1220, 1991
23. Cassatella MA, Meda L, Bonora S, Ceska M, Constantin G: Interleukin 10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumor necrosis factor and IL-1β in mediating the production of IL-8 triggered by lipopolysaccharide. J Exp Med 178:2207–2211, 1993
24. Kasama T, Strieter RM, Lukacs NW, Burdick MD, Kunkel SL: Regulation of neutrophil-derived chemokine expression by IL-10. J Immunol 152:3559–3569, 1994
25. Sieling PA, Abrams JS, Yamamura M, Salgame P, Bloom BR, Rea TH, Modlin RL: Immunosuppressive roles for IL-10 and IL-4 in human infection: *In vitro* modulation of T cell responses in leprosy. J Immunol 150:5501–5510, 1993
26. Chomarat P, Rissoan M-C, Banchereau J, Miossec P: Interferon gamma inhibits interleukin 10 production by monocytes. J Exp Med 177:523–527, 1993
27. Del Prete G, Carli MD, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S: Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 150:353–360, 1993
28. De Waal Malefyt R, Yssel H, De Vries JE: Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells. Specific Inhibition of IL-2 production and proliferation. J Immunol 150:4754–4765, 1993
29. Taga K, Tosato G: IL-10 inhibits human T cell proliferation and IL-2 production. J Immunol 148:1143–1148, 1992
30. Ralph P, Nakoinz I, Sampson-Johannes A, Fong S, Lowe D, Min H-Y, Lin L: IL-10, T lymphocyte inhibitor of human blood cell production of IL-1 and tumor necrosis factor. J Immunol 148:808–814, 1992

31. Miller CL, Graziano CJ, Lim RC: Human monocyte plasminogen activator production: Correlation to altered MØ-T lymphocyte interaction. J Immunol 128:2194–2200, 1982
32. Miller-Graziano CL, Fink M, Wu JY, Szabo G, Kodys K: Mechanisms of altered monocyte prostaglandin E_2 production in severely injured patients. Arch Surg 123:293–299, 1988
33. Szabo G, Miller-Graziano CL, Wu J-Y, Takayama T, Kodys K: Differential tumor necrosis factor production by human monocyte subsets. J Leukocyte Biol 47:206–216, 1990
34. Looney RJ, Abraham GN, Anderson CL: Human monocytes and U937 cells bear two distinct Fc receptors for IgG. J Immunol 136:1641–1647, 1986
35. Fox FE, Ford HC, Douglas R, Cherian S, Nowell PC: Evidence that TGF-β can inhibit human T-lymphocyte proliferation through paracrine and autocrine mechanisms. Cell Immunol 150:45–58, 1993
36. Geppert TD, Lipsky PE: Activation of T lymphocytes by immobilized monoclonal antibodies to CD3 regulatory influences of monoclonal antibodies to additional T cell surface determinants. J Clin Invest 81:1497–1505, 1988
37. Miller-Graziano CL, Szabo G, Griffey K, Mehta B, Kodys K, Catalano D: Role of elevated monocyte transforming growth factor β (TGFβ) production in post-trauma immunosuppression. J Clin Immunol 11:95–102, 1991
38. De AK, Kodys K, Fairfield S, Miller-Graziano C: Relationship of post-trauma altered IL-12 and IL-10 to depressed patient mitogen responses. *In* Host Defense Alterations of Trauma, Shock and Sepsis—Multiorgan Failure/Immunotherapy of Sepsis, E Faist (ed). Berlin, Springer-Verlag, 1994, in press
39. Wanidworanun C, Strober W: Predominant role of tumor necrosis factor-α in human monocyte IL-10 synthesis. J Immunol 151: 6853–6861, 1993
40. Marchant A, Deviere J, Byl B, de Groot D, Vincent J-L, Goldman M: Interleukin-10 production during septicemia. Lancet 343:707–708, 1994
41. Frei K, Lins H, Schwerdel C, Fontana A: Antigen presentation in the central nervous system. The inhibitory effect of IL-10 on MHC class II expression and production of cytokines depends on the inducing signals and the type of cell analyzed. J Immunol 152: 2720–2728, 1994
42. Bogdan C, Paik J, Vodovotz Y, Nathan C: Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor-β and interleukin-10. J Biol Chem 267: 23301–23308, 1992
43. Szabo G, Kodys K, Miller-Graziano CL: Elevated monocyte interleukin-6 (Il-6) production in immunosuppressed trauma patients. I. Role of FcγRI crosslinking stimulation. J Clin Immunol 11:326–335, 1991
44. Miller-Graziano CL, Szabo G, Kodys K, Griffey K: Aberrations in post-trauma monocyte subpopulation: Role in septic shock syndrome. J Trauma 30:S86–S97, 1990
45. Ishida H, Hastings R, Thompson-Snipes L, Howard M: Modified immunological status of anti-IL-10 treated mice. Cell Immunol 148:371–384, 1993
46. Mulligan MS, Jones ML, Vaporciyan AA, Howard MC, Ward PA: Protective effects of IL-4 and IL-10 against immune complex-induced lung injury. J Immunol 151:5666–5674, 1993
47. Pradier O, Gérard C, Delvaux A, Lybin M, Abramowicz D, Capel P, Velu T, Goldman M: Interleukin-10 inhibits the induction of monocyte procoagulant activity by bacterial lipopolysaccharide. Eur J Immunol 23:2700–2703, 1993
48. Teodorczyk-Injevan JA: Activation-related T cell anergy after thermal injury. Intensive Care Med 20:S77, 1994
49. Szabo G, Verma B, Mandrekar P, Catalano D: Acute ethanol uptake prior to injury modulates monocyte TNFα production and mononuclear cell apoptosis. Intensive Care Med 20:S82, 1994
50. Hall SS: Immune therapies. IL-12 holds promise against cancer, glimmer of AIDS hope. Science 263:1685–1686, 1994
51. Fluckiger A-C, Garrone P, Durand I, Galizzi J-P, Banchereau J: Interleukin 10 (IL-10) upregulates functional high affinity IL-2 receptors on normal and leukemic B lymphocytes. J Exp Med 178:1473–1481, 1993
52. Taga K, Chretien J, Cherney B, Diaz L, Brown M, Tosato G: Interleukin-10 inhibits apoptotic cell death in infectious mononucleosis T cells. J Clin Invest 94:251–260, 1994