Association of a Circulating Immunosuppressive Polypeptide with Operative and Accidental Trauma

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The serum from 109 traumatized patients was examined for immunosuppressive activity which might explain diminished host immune responsiveness following operative or accidental injury. Twenty-eight of 31 (90%) severely traumatized patients, 25 of 60 (42%) moderately traumatized patients, and 0 of 18 minimally traumatized patients developed serum which suppressed the response of normal human lymphocytes to phytohemagglutinin. The degree and duration of serum immunosuppressive activity paralleled the severity of the clinical course but did not correlate with serum cortisol or barbiturate levels. Suppressive sera were not cytotoxic. The immunosuppressive factor(s) was contained in a low molecular weight (<10,000 daltons) peptide fraction and was present in 5–10 times the amount recoverable from normal serum. By size and activity the trauma serum factor resembled immunoregulatory alpha globulin, a naturally-occurring serum inhibitor of T-lymphocyte reactions. Thus, depressed immunoreactivity following trauma may be due in part to high concentrations of an endogenous immunosuppressive polypeptide.

THERE is considerable clinical and experimental evidence that operative and accidental trauma decrease host immune responsiveness. The basis for such impairment, however, is not established. Previous studies which demonstrate transient depression of serum immunoglobulin or complement levels following thermal burns[2,10,19,40] and surgery[36] do not account for the magnitude and duration of post-traumatic immunosuppression and fail to explain the profound impairment of cellular immunity under these circumstances. Susceptibility to bacterial, viral, and mycotic infection greatly increases following thermal injury[1] and surgery.[14,43,45] Similarly, skin allograft[18] and xenograft[37] survival are prolonged, the incidence of experimental tumor metastasis increases[5,20] and normal reactivity to skin test antigens decreases or disappears.[6,22] Further, macrophage[46] and lymphocyte[38,44] function are temporarily depressed following trauma; function recovers, however, if the cells are tested in normal serum, suggesting that their hyporeactivity may have resulted from cell coating by a serum component.

For a number of years our laboratories have been involved in the purification and characterization of a naturally-occurring immunosuppressive material in human serum, designated immunoregulatory alpha globulin (IRA). IRA was originally recovered as a protein subfraction from alpha globulin-rich Cohn Fraction IV,[23] but was later demonstrated to be a low molecular weight peptide(s) associated with, or carried by, the alpha globulins.[34] IRA inhibits a wide variety of T-lymphocyte-mediated immunologic reactions, including the rejection of skin[23] and whole organ allografts[26] and transplantable tumors,[13] and the stimulation of peripheral blood lymphocytes by thymus-dependent antigens and by phytohemagglutinin.[8,27] Further, IRA decreases phagocytosis[11] and resistance to experimental bacterial infection.[12] Such data, and the observation by Viet and Michael[48] and others[3,28,39,47] that IRA-like activity in the serum is increased during immune stimulation involving T-lympho-

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TABLE 1. *Patients Studied*

No. Patients	Source of Trauma	Age
Class I*		
1	Repair urethral diverticulum	32
2	Cystoscopy	50, 67
2	Dilatation and curettage	21, 30
2	Uncomplicated vaginal delivery	23, 35
2	Closed fracture	16, 19
2	Minor penetrating chest trauma	27, 38
3	Mild blunt abdominal trauma	33–49
2	Minor penetrating abdominal trauma	30, 32
2	Minor blunt head trauma	16, 35
Class II		
25	Coronary artery bypass	40–63
2	Major blunt chest trauma	16, 30
4	Excision abdominal aortic aneurysm	27–60
3	Cardiac valve replacement	34–61
1	Subtotal colectomy (ulcerative colitis)	40
3	Pulmonary lobectomy (actinomycosis, empyema, pulmonary abscess)	51–56
1	Esophageal bypass (stricture)	50
3	Transurethral resection of prostate (benign hypertrophy)	68–77
1	Splenectomy	50
1	Division of small bowel adhesions	33
1	Hemigastrectomy	55
1	Right hepatic lobectomy (trauma)	24
3	Peripheral vascular surgery	35–65
1	Cystopexy	56
2	Perforated duodenal ulcer	32, 40
2	Craniotomy (chronic subdural hematoma)	55, 65
1	Incarcerated inguinal hernia	76
5	Thermal burn	35–50
Class III		
7	Coronary artery bypass	45–65
2	Acute hemorrhagic pancreatitis	42, 66
1	Ruptured abdominal aortic aneurysm	60
1	Acute appendicitis with perforation	60
4	Excision abdominal aortic aneurysm	40–83
1	Multiple skull fractures	50
1	Pancreatic pseudocyst	66
3	Perforated duodenal ulcer	67–75
1	Chronic subdural hematoma	86
1	Mallory-Weiss syndrome	54
9	Thermal burn	3–48

* Class I patients underwent minor trauma; Class II patients underwent major trauma; Class III patients underwent major trauma with life-threatening complications.

cytes suggested that IRA may function as a regulator of T-cell-mediated immunity. Overactivity of the normal feedback mechanism with excessive production of IRA might account for impaired post-traumatic cellular immunity and should be reflected by the appearance of immunosuppressive serum and high concentrations of circulating IRA-like polypeptide following injury.

In the present study we have observed that the serum of operatively or accidentally traumatized patients is immunosuppressive but not cytotoxic. This immunosuppressive activity appears to be due in part to the presence of abnormally large amounts of a low molecular weight serum peptide which resembles IRA-peptide in its in vitro activity.

Methods

Serum collection

Three hundred forty-seven serial blood samples were drawn from 109 patients before and subsequent to varying degrees of operative or accidental trauma. Informed consent was obtained from each donor. Control sera were obtained from healthy medical school personnel. In addition, preoperative samples were taken from 15 patients prior to elective surgery; hence, the patient served as his own control. Since some inhalation anesthetics may be immunosuppressive[4,9,17] controls were included for this variable.

Blood samples were allowed to clot, and then centrifuged at 400 × g for 10 minutes. The serum was pipetted into sterile, untreated glass tubes and stored at −4°. Immunosuppressive activity was tested at a concentration of 10% as described below.

Patients Studied

Patients were selected to provide a wide range of diseases or injuries (Table 1). No patients known to have malignant neoplasms were included. Patients were divided according to the severity of trauma: Class I, minor trauma; Class II, major trauma with an uncomplicated clinical course; Class III, major trauma with life-threatening complications.

Serum Fractionation

Fractionation was carried out by diethylaminoethyl (DEAE) cellulose column chromatography as described recently[33] using gradient and stepwise elution with increasing ionic strength acetate buffers at pH 5. The resulting 6 protein peaks were individually desalted by gel filtration on G-25 Sephadex and tested for immunosuppressive activity as outlined below in doses of 0.75–6 mg/ml of medium. The peptide fraction was obtained by acidification of the first DEAE protein peak and ultrafiltration with a membrane which retained species of molecular weight greater than 10,000 daltons.[33] The peptide fraction passing through the filter was lyophilized and tested for immunosuppressive activity in doses of 0.03 to 0.6 mg/ml of medium.

Determination of Immunosuppressive Activity

Immunosuppressive activity was defined as 50% or greater inhibition of the uptake of tritiated thymidine by phytohemagglutinin (PHA)-stimulated normal human lymphocytes, using a tissue culture method described earlier.[33] Test serum or serum fractions were added to culture medium simultaneous with PHA. Per cent suppression was calculated from the ratio of radioactivity

TABLE 2. *Suppression of PHA-stimulation of Normal Human Lymphocytes by Trauma Serum*

Patient Class	No.	No. Suppressive	% Suppressive
I	18	0	0%
II	60	25	42%
III	31	28	90%
Total II + III	91	53	54%

(counts per minute, cpm) in tubes containing test serum to tubes containing a 10% concentration of homologous AB-serum:

$$\text{Per cent Suppression} = \left(1 - \frac{\text{cpm in experimental tubes} - \text{cpm of unstimulated tubes}}{\text{cpm of stimulated tubes} - \text{cpm of unstimulated tubes}}\right) \times 100$$

For purposes of comparison, inhibitory activity of the protein fractions was designated in immunosuppressive units, defined as the ratio of the total weight of the protein fraction to the smallest dose producing complete *in vitro* suppression of the lymphocyte response to PHA.

Cytotoxicity of test fractions was measured by trypan blue dye exclusion.

Chemical Determinations

Cortisol was measured by the method of Murphy.[32] Barbiturate levels were performed by Leary Laboratories, Inc., Boston, using a gas chromatographic method. The polypeptide moiety was determined by the method of Mehl[25] using bovine serum albumin as a standard.

Results

Whole Serum Suppressive Activity

Twenty-eight of 31 patients (90%) developed immunosuppressive serum after undergoing major operative or accidental trauma with life-threatening complications (Class III, Table 2); 25 of 60 (42%) patients undergoing major trauma without complications (Class II) developed immunosuppressive serum; 0 of 18 patients who underwent minor trauma (Class I) developed suppressive serum. Differences between Classes I and II, I and III, and II and III were significant ($P < 0.005$) by the Chi-Square Test (Yates' correction). Sera from 15 normal controls were not suppressive, and none of 14 samples from patients receiving anesthesia without major surgery was suppressive (e.g., Class I patients cystoscoped or undergoing uncomplicated vaginal delivery under general anesthesia). In addition, 10 of 11 patients who sustained major accidental trauma had immunosuppressive serum as early as one hour following trauma and before receiving anesthesia. While there was measurable barbiturate in the serum or serum fractions of postoperative patients who had received barbiturates preoperatively or intraoperatively, high barbiturate levels did not parallel high serum or serum fraction immunosuppressive potency. Finally, none of 15 patients on whom pre-trauma samples were drawn had immunosuppressive serum before trauma.

TABLE 3. *Degree of Suppression of PHA-stimulation by Trauma Serum*

	>70%		>90%	
Patients	No.	%	No.	%
Class II	18/25	72	11/25	44
Class III	23/28	82	19/28	71

Among those patients who developed suppressive serum the immunosuppressive potency of the serum paralleled the severity of the trauma (Table 3). These differences between Classes II and III are significant ($P < 0.05$) by the Chi-Square Test.

The time course for the appearance and disappearance of whole serum suppressive activity is shown in Table 4.

TABLE 4. *Degree and Duration of Serum Suppressive Activity Following Trauma**

		Days after Trauma					
Patients	Pre-Trauma	1	2	3–5	6–8	9–14	15–28
Class III	20 ± 20† (2)‡	75 ± 4 (36)	69 ± 7 (14)	69 ± 6 (18)	72 ± 8 (8)	71 ± 6 (18)	66 ± 6 (11)
Class II	25 ± 7 (9)	60 ± 4 (65)	64 ± 14 (6)	52 ± 13 (6)	30 ± 4 (2)		
Class I	14 ± 2 (4)	36 ± 5 (17)	22 (1)	18 ± 2 (2)	10 (1)		

* Mean percent suppression ± S.E.

* Analysis of Variance followed by a test for all possible comparisons among means shows that each class is different from each other class ($P < .01$) and that pre-trauma is different from 1, 2, 3–5, 6–8, 9–14, and 15–28 days after trauma ($P < .01$). The days after trauma do not differ from each other.

‡ Number of samples.

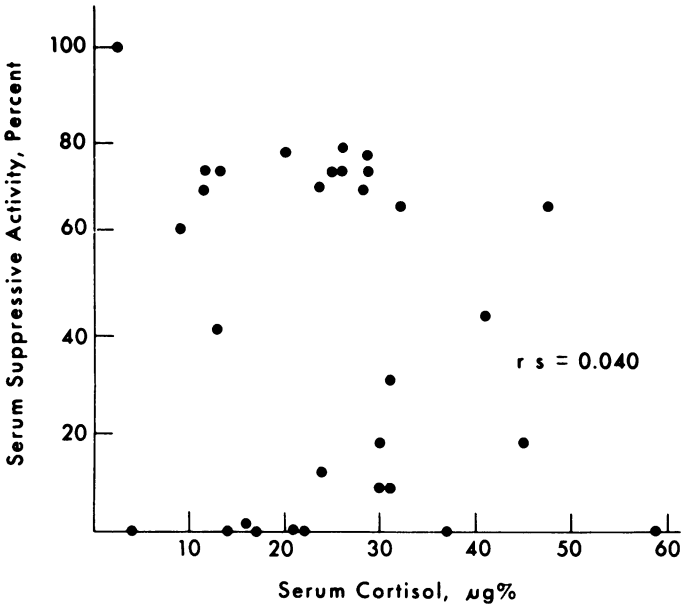

FIG. 1. Scattergram of cortisol levels and immunosuppressive activity of 31 trauma serum samples.

For all those patients undergoing major trauma who developed immunosuppressive serum, inhibitory activity was often detectable immediately (i.e., as early as one hour) following trauma and slowly decreased over a variable length of time depending on the severity of the clinical course. Further, the degree and duration of suppression of PHA-stimulation paralleled the severity of illness.

There was no correlation ($r_s = 0.040$) between cortisol levels and immunosuppressive activity for 31 trauma serum samples (Fig. 1).

Trauma patient serum was not cytotoxic in the doses employed as judged by trypan blue dye exclusion.

Immunosuppressive Activity in Serum Fractions

Immunosuppressive units for the 7 protein fractions developed by DEAE-cellulose serum chromatography are given in Table 5. The data represent 43 individually fractionated serum samples: 20 from 15 Class II patients, 18 from 9 Class III patients, and 5 from 5 normal volunteers. It is clear that the first protein peak (Fraction I) from trauma serum contains considerable immunosuppressive activity not present in the serum of healthy volunteers. The remaining peaks (II–VII) contain smaller amounts of material, of comparable magnitude in all groups. There is also clearly more total immunosuppressive material yielded by trauma serum, being 5 to 10 times that recoverable from the normal serum tested.

DEAE Fraction I contained 80–90% of the total immunosuppressive material; the dose-related inhibition of PHA-stimulation is given in Table 6. Further, the number of immunosuppressive units recovered from Fraction I of each class of patients paralleled the severity of trauma; thus Class III serum yielded 288 ± 82.3 units, while Class II and I serum yielded 195.4 ± 29.2 and 136.0 ± 38.7 units, respectively. Fraction I protein was not lymphotoxic in the tested doses as judged by trypan blue dye exclusion.

When serial serum aliquots obtained before and subsequent to trauma were chromatographed (Fig. 2), the duration and amount of Fraction I immunosuppressive activity paralleled the clinical course, as was previously observed for whole serum suppression.

Since initial studies has indicated that Fraction I contained the greatest amount of suppressive material, a pool of 18 immunosuppressive Fraction I protein samples was acidified to pH 3.0 and diafiltered with a membrane which retained species greater than 10,000 daltons. The procedure has been detailed earlier[33] and was similar to procedures used to dissociate IRA-peptide from its larger molecular weight (probably alpha globulin) protein carrier.[34] The results are given in Table 7. Unfiltered Fraction I protein from which the peptide was extracted was 99.7% suppressive of PHA-stimulation at 1.5 mg/ml. The fraction recovered by filtration was 99.5% suppressive at 0.3 mg/ml, while the material retained by the filter was no longer suppressive below 6.0 mg/ml. The ultrafiltrate of normal serum possessed no activity even at 6.0 mg/ml. Analysis by the biuret method indicated that the total weight of the immunosuppressive low molecular weight fraction could be accounted for by polypeptide. The peptide fraction added to stimulated or unstimulated cultures was not lymphotoxic by trypan blue dye exclusion.

TABLE 5. *Immunosuppressive Units for Individual Protein Fractions Developed by DEAE-fractionation of Trauma Serum*

Fraction	I	II	III	IV	V	VI	VII
Class II patients (n = 15)	195.4 ± 29.2*	0	10.0 ± 6.1	11.5 ± 6.5	3.2 ± 2.0	14.9 ± 8.0	13.2 ± 5.7
Class III patients (n = 9)	288 ± 82.3	—	45 ± 6.5	—	—	—	—
Normal volunteers (n = 5)	0	0	8.0 ± 4.2	10.0 ± 8.1	24.2 ± 10.0	15 ± 4.0	8.0 ± 3.4

* Mean immunosuppressive units ± S.E.

TABLE 6. *Inhibition of PHA-stimulation by Fraction I Protein from DEAE Chromatography of Trauma Serum*

Fraction I Protein	6 mg/ml	3 mg/ml	1.5 mg/ml
Trauma patients (n = 24)	94.4 ± 2.3*	85.9 ± 3.7	74.8 ± 5.2
Normal volunteers (n = 8)	11.0 ± 5.5	34.2 ± 16.0	31.2 ± 20.3

* Mean per cent suppression ± S.E.

Discussion

The prevalence of lethal infectious complications after operative or accidental trauma strongly supports the concept of diminished host immune reactivity in this circumstance, although the mechanism has not been established. Traumatized hosts do not manifest a normal immune response to skin tests, allografts, tumor growth, or viral and mycotic infection, all of which depend in part upon intact T-cell function. Several reports[7,15,35,44] indicate that lymphocytes of postoperative patients are hyporesponsive to mitogenic stimulation; such diminished reactivity often occurs only in autologous, but not pooled, serum[22,38,44] suggesting that the lymphocytic hypofunction may not be intrinsic but related to some characteristic of the serum. In fact, the serum from burned patients has been shown to depress the response of normal lymphocytes to Monilia antigen.[31] The active factor was not cortisol. Further, there have been reports that "convalescent serum"[16,24] reduces the mortality from infection in burned animals, which may reflect interference with, or dilution of, the immunosuppressive serum factor(s). Finally, a number of reports have suggested that impairment of cellular immunity following surgical[15,20,21,29,42] or thermal[30,37,42] trauma is transient and may be related to the severity of the injury. Clearly, the presence of a circulating immunosuppressive factor which antagonizes T-cell activity would account for much of the observed functional immune deficit following operative or accidental injury.

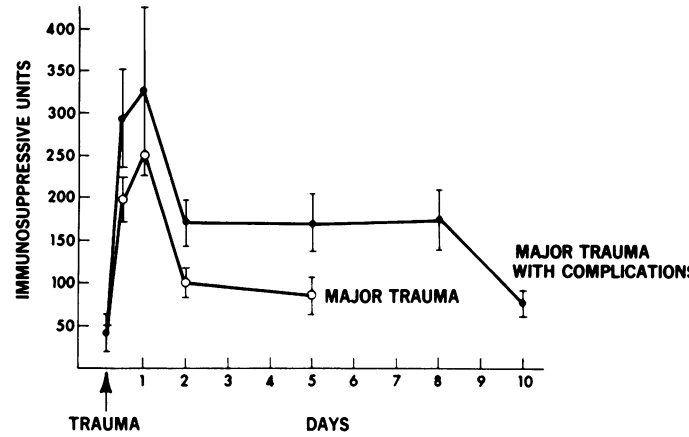

FIG. 2. Immunosuppressive material recoverable from patient serum after operative or accidental trauma; points represent mean ± S.E.

IRA has previously been shown to inhibit a number of T-cell dependent immune phenomena without toxicity,[27] probably by interference with antigen recognition. Specifically, IRA depresses the primary antibody response to antigens which depend upon T-cell helper activity.[8,27] IRA inhibits the blastogenic response of normal human lymphocytes to mitogens, including PHA.[27] Pretreatment of rodents with IRA impairs rejection of skin[23] and kidney allografts[26] and transplantable syngeneic tumors.[13] IRA depresses resistance to experimental streptococcal infection.[12] Presumably, IRA blocks immune responses by low-affinity binding to the lymphocyte plasma membrane, since the suppressive effect can be easily removed by washing.[8] Excessive amounts of this naturally-occurring suppressor following trauma would be expected to cause widespread impairment of cellular immunity.

The experiments described here indicate that the sera of traumatized patients contains immunosuppressive activity which parallels the severity of the clinical course. We have not established whether whole serum immunosuppression is causative or predictive, i.e., if the Class III patients suffered major complications because they

TABLE 7. *Comparison of Immunosuppressive Activity of Fraction I "Starting Material" and Materials Retained by, and Passing Through the PM-10 Ultrafilter*

	Dose (per ml)					
	6 mg	3 mg	1.5 mg	0.3 mg	0.1 mg	0.03 mg
Starting fraction I protein	100 ± 0*	100 ± 0	99.7 ± 0.3	—	—	—
Retained protein fraction	96.0 ± 4.0	3.3 ± 3.3	0	—	—	—
Filtered peptide fraction	—	—	—	99.5 ± 0.5	48.0 ± 27.7	12.5 ± 12.5

* Mean per cent suppression ± S.E.

were immunosuppressed, or whether their serum merely reflected qualitatively the degree of their illness. MacLean[22] has presented data which indicate that preoperative cutaneous anergy is predictive of a high incidence of postoperative infectious complications, and has found suppressive serum activity in 4 of 4 severely ill patients tested.

Column chromatography of the serum yielded a protein fraction (Fraction I) whose specific activity paralleled the severity and duration of the clinical course. Neither whole serum nor Fraction I protein suppressivity could be correlated with serum cortisol or barbiturate levels. Trauma serum yielded 5 to 10 times the amount of immunosuppressive material recoverable from control serum.

Finally, ultrafiltration of Fraction I protein indicated that a majority of the immunosuppressive activity was contained in a peptide subfraction of low (<10,000 daltons) molecular weight. By size, and by its ability to inhibit PHA-stimulation, this peptide resembles IRA.

It is possible, but by no means certain, that the suppressive trauma serum peptide represents large quantities of the naturally-occurring IRA. However, the origin of both materials is unclear. We have suggested that IRA is a feedback regulator of T-cell mediated immunity, and may be produced by suppressor lymphocytes during immune stimulation. In the traumatized host, chronic antigenic stimulation or some other more obscure mechanism may result in the production of large amounts of IRA; such reasoning is supported by our finding of greater amounts of suppressive activity in the more severely ill patients. It is possible that the failure of the cellular immune response to protect patients following severe operative or accidental injury may result in part from overreaction of a normal immunoregulatory mechanism.

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