MEDIATORS AFFECTING IL2 FUNCTION IN BURN IMMUNOSUPPRESSION

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INTRODUCTION

Thermal injury induces the most complex array of physiological dysfunction known. Extensive research effort has led to an understanding of some of the changes occurring following a major burn, yet early pathological changes have been documented to be different from those of later phases and great difficulties have arisen in determining cause-and-effect relationships in these changes. For example, intensive first treatment using specific, systemic and local therapy successfully delays mortality in the early phase of very severe burns but has little effect on the outcome. This was observed where burn victims, treated early, survived initially in greater numbers than those devoid of early treatment (48% greater at day 4, 32% at day 12), whereas the difference was only 7.3% at 2 months [1]. The fatal outcome of the late phase is ascribed to multiple organ system failure (MOSF) but not always to concomitant sepsis, for often bacteria

cannot be detected in up to half the patients who die [2]. Where sepsis had been well controlled and was not confirmed at death, it had been surmised that infection was not the primary cause of death. In this situation, devitalized tissue and/or circulating endotoxin have been thought to perpetuate some mediator-induced response which lead to MOSF[2]. Evidence from animal models suggests, however, that endotoxic shock and burn shock are two different pathologies. Whereas endotoxin effects are mediated through lipid peroxidation and can be reversed by catalase and superoxide dismutase, burn shock is not protected by these enzymes [3]. Thus even when endotoxins are present they may not be the main mediators of late death in burns.

One significant system which can undergo failure is the immune system, specifically the T cell system. It has been shown that the degree of T cell failure (anergy) correlates well with mortality rates [4], but there is a continuing debate over whether anergy causes sepsis or is caused by sepsis. Early studies on T cell function in burn injury [5,6] ascribed the failure to an activation of non-specific suppressor cells, since cocultures of normal lymphocytes with burn patients' lymphocytes gave lower than normal responses. In these studies bacterial endotoxin was claimed to mediate the activation of suppressor cells [7], as it was known that polyclonal B cell activators could lead to generation of suppressor lymphocytes [8]. In particular, suppression was claimed to involve the macrophage which releases prostaglandin E (PGE) and interleukin-1 (IL1) upon stimulation with endotoxin.

PGE would then induce the suppressor T cell [9]. However in burn patients' circulating cells, IL1 levels proved to be more or less in the normal range up to 50 days after the burn, and PGE levels in plasma did not correlate with immune suppression [10]. Thus, the question of endotoxin-mediated suppressor cell activation in burn trauma may be factitious, especially as a result of direct measurements of the suppressor lymphocyte marker OKT8. After the first week, numbers of post burn OKT8-positive cells were shown to be normal, and the low T4/T8 ratio was due to a decrease in number of T4 helper lymphocytes [11,12].

Our analysis of burn patient's humoral and cellular immune status revealed an abnormally high immunoglobulin (Ig) production *in vitro* in the first few weeks, followed by a depressed response which was either transient in survivors, or permanent in non-survivors. These changes did not correlate well with the proliferative responses to conventional mitogens [13] but were well correlated with the allogenic responses in the mixed lymphocyte reaction (MLR) [14,15] suggesting T helper cell functional failure.

We further observed that production of both IL2 and its receptor (IL2R) in burn patient's lymphocytes was markedly suppressed during or shortly before the functionally observed immunosuppression [16,17]. IL2R levels decreased over the post burn period, but if they remained above 50% of the first day level they increased again leading to survival. Once below 50% of the first day level, expression of IL2R continued to drop until death. Addition of IL2 to survivors' cell

cultures increased both the number of IL2R and the MLR response. In non-survivors' cells, IL2 also raised the IL2R levels, but did not reverse the low MLR functional response. Thus, terminal burn injury appears to affect the expression of IL2 functional receptors [18].

This report deals with our preliminary examination of IL2 function and factors implicated in post burn immunosuppression, such as post burn serum, PGE_2, and a very well characterized lipid-protein complex induced in skin by burning (Cutaneous Burn Toxin, CBT). This toxin is known to depress the *in vivo* immune response to *Pseudomonas* [19] and the *in vitro* lymphocyte proliferative response [20].

MATERIALS & METHODS

Preparation of peripheral blood mononuclear cells (PBMC), culture conditions and enumeration of IL2R-expressing cells by flow cytometry, were performed as described in detail elsewhere [17]. Assessment of immunoglobulin (Ig) production in pokeweed mitogen stimulated PBMC cultures was performed as described before [15]. Post burn sera were tested at a concentration of 10% (v/v), IL2 (Biogen Corp.) was used at 20U/ml, PGE_2 (Sigma Co.) at 10^{-6} to 10^{-8}M and indomethacin (Sigma Co.) at a concentration of 1μg/ml. CNBr-activated Sepharose 4B (Pharmacia) and monospecific anti-PGE_2 serum (Sigma Co.) were utilized for the affinity chromatography adsorption of post-burn sera. CBT was

isolated and purified from burned human skin following the procedures of Dr. G. Schoenenberger [21] in his laboratory. Antiserum to CBT was produced in sheep and the immune globulin fraction was provided by Dr. G. Schoenenberger. The human IL2-dependent cell line [22] was provided by Dr. R. M. Gorczynski of the Ontario Cancer Institute, Princess Margaret Hospital, Toronto. These cells were cultured at 10^5/ml in microwell plates in volumes of 0.1 ml with tritiated thymidine (1μCi in 50μl) added at the 48^{th} hour. Plates were harvested at the 64^{th} hour. Standard IL2, when added in 50μl, was in a dilution calculated to promote proliferation registering about 4000 cpm.

RESULTS

IL2-bearing cells were enumerated in ConA-stimulated PBMC from normal controls and from burn patients at day 1 and at the time of maximal immunosuppression which varied from day 10 to day 40. IL2 added to these cultures increased the percentage of IL2R-positive cells in all cultures (Table 1). The addition of indomethacin (IM) to cultures had no effect on normal receptor levels or on receptor expression at the time of immunosuppression; but it did increase the expression on cells isolated on the first day of the injury.

When PGE_2 was added to cultures of normal PBMC in the presence of normal human serum (NHS), there was a drop in percentage of IL2R-positive cells. IL2 in these cultures reversed the inhibition caused by PGE_2 (Table 2). Post burn

(PB) serum isolated on day 1 was not suppressive of IL2R expression, but serum isolated at the time of immunosuppression (days 10 to 40) did show IL2R suppression. Removal of PGE_2 from immunosuppressive serum, using antibody to PGE_2, did not completely reverse the inhibition. These results suggested that immunosuppressive sera contain factors other than PGE_2 which affect IL2 receptor expression.

The toxin from burned skin is known to appear in patient plasma increasingly after thermal injury [23]. To study the viability of PBMC in the presence of CBT, cultures of 10^6 cells/ml were established with CBT ranging from 0.25 to 10 μg/ml. The percent viable cells after 48 hours was enumerated by trypan blue staining and the numbers of cells per ml were counted using the Coulter Counter. CBT had no effect on the viability of PBMC (Table 3). As a test for its effect on immunoglobulin production, CBT was incubated *in vitro* with 5 x 10^5 PBMC/ml and pokeweed mitogen at 10μg/ml. From 0.15 to 0.6 μg/ml, CBT did not inhibit IgG production but 1.25 and 2.5 μg/ml concentrations were suppressive (Fig 1). Supernatants from cultures of 10^6 cells/ml incubated in the presence of 5μg/ml phytohemagglutinin, and with CBT ranging from 0.12 to 10μg/ml, produced decreasing amounts of IL2. The IL2 was assayed by the growth of an IL2-dependent human T cell line (data not shown). The direct effect of CBT on proliferation of this IL2-dependent cell line, in the presence of a standard amount of IL2, was to inhibit the growth increasingly as the concentration of CBT increased

(Fig 2). Fifty percent of the optimal growth occurred in the presence of about 1.0μg/ml CBT in this system.

DISCUSSION

Some laboratories have recently focused on the failure of IL-2 function in T lymphocytes of severely burned patients [10,11,17]. Since it is known that PGE inhibits the production of human IL2 [9], we tested the sensitivity of lymphocytes from the burn patients to induction of receptors for IL2, in the presence of indomethacin. At the time of the patients' immunosuppression (days 10 to 40), the decreased percentage of IL2R-bearing cells was not augmented by IM. On the first day, however, IM did augment the percentage of IL2R-positive cells. This indicated that endogenous PG production in PBMC was active on the first day but not at the time of immunosuppression. A biphasic release of eicosanoid metabolites in burn patients was reported [24] to be very high on day 1 and was then exhausted. Our findings are consistent with this pattern. Thus, the immunosuppression of the later period, characterized by reduction in helper cell numbers and in IL2 function, was not dependent on endogenous eicosanoid metabolism.

When PGE_2 was added to cell cultures of normal subjects, in concentrations known to be immunosuppressive *in vitro,* it reduced the percentage of IL2R-positive cells. However, this reduction was reversed in the presence of IL2. Serum

from patients on the first post-burn day did not suppress levels of IL2R expression, but at the time of immunosuppression burn serum did lower the percentage of IL2R-positive cells. However, the addition IL2, and the removal of PGE_2 both brought the percentages of IL2R-positive lymphocytes up to similar but below normal levels. This suggested that factors affecting IL2R, other than PGE_2, might exist in the immunosuppressive serum.

In recent years, immunosuppressive serum factors have been gaining attention. They are reported to be of a variety of molecular sizes [10, 24, 25, 26]. The large lipid-protein complex from burned skin, CBT, was known to have immunosuppressive properties [19,20]. CBT appears in the plasma of burn patients at concentrations ranging from 10 to 100 μg/ml [23], and seems to peak at about the sixth day post-burn [27]. When it was incubated with PBMC, to observe its effect on cell viability, no deleterious effects were noted *in vitro* in 48 hours. However in assays of immune function, CBT at 1.25 and 2.5 μg/ml caused a marked inhibiton of PWM-induced IgG production by PBMC at 5×10^5/ml. As well, PHA-induced IL2 production was arrested. The complex, at about 1.0 μg/ml, directly inhibited 50% of the proliferative response of a human IL2-dependent cell line cultured initially at 10^5 cells/ml in the presence of IL2. It is not yet known whether CBT exerted its effect by binding IL2, or had a direct effect on the membrane IL2R.

Proper IL2 function now appears to be critical to the late burn mortality, as non-survivors' lymphocytes cannot respond to exogenous IL2 [18]. CBT had earlier been implicated in late burn mortality [28], because CBT plasma levels correlated with patient death, to a highly significant degree[19]. Therefore it is a significant finding that CBT interferes with a mechanism so critical to recovery from thermal injury. Curiously, early eschar excision and wound closure, involving extensive surgery and multiple anaesthesics, are procedures which themselves should be immunosuppressive. Yet they are known to improve the immunologic depression profoundly [29] as demonstrated in animal models [30,31]. Thus, evidence exists which links thermal injury of skin to an immune failure in severe burns. Since skin has an immunological function [32] CBT may be a complex of some immunoreactive material in skin, perhaps reacting with IL2 or its receptor. However, immunosuppression mediated by CBT will have to be weighed in consideration with other immunosuppressive factors.

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Table 1. Effect of IL2 (20U/ml) and indomethacin (1 μg/ml) on IL2R expressed by ConA-stimulated PBMC from normal donors and burn patients.

Culture Conditions	% IL2R-positive cells at 72 hr			
	Burn Patients			Controls
	Day 1	Day 10-40		
		Surviving	Non-surviving	
PBMC	26.1±6.3	16.6±8.1	<5	31.4±12.5
PBMC + IL2	37.9±8.2	34.2±11.2	15.5±3.1	54.1±5.3
PBMC + indomethacin	41.7±7.9	14.3±7.0	<5	33.4±8.5

Table 2. Effect of post-burn (PB) sera (10%) and IL2 (20U/ml) on expression of IL2R in ConA-stimulated cultures of normal PBMC*

Serum	Expression of IL2R after 72 hr [% of control level]§	
	without IL2	with IL2
NHS, pooled	100	145.9
PB, day1	117.2	135.7
PB, day 10-40	26.0	74.8
PB, day 10-40/anti-PGE_2	76.0	108.9
NHS + PGE_2 10^{-6}M	50.4	77.2
NHS + PGE_2 10^{-7}M	46.3	101.6
NHS + PGE_2 10^{-8}M	52.8	105.7

*Table 2 presents the means of results from 7 separate experiments in which 69 individual serum samples collected from burn patients 1-40 days post-burn were tested.

§ Results are expressed as a percentage of the IL2R-bearing cells enumerated in ConA-activated cultures incubated in the presence of normal human serum (NHS, pooled).

Table 3. Effect of CBT on growth and viability of PBMC cultured at 37 ° C for 48 Hrs.

	CBT Concentration (μg/ml)							
	0	0.25	0.5	1.0	2.5	5	10	
Cell concentration (X 10^{-6}/ml)								Mean
0 Hrs:	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
48 hrs:	1.73	1.76	1.63	1.86	1.68	1.87	1.80	1.77±0.10
Viability %								
0 Hrs:	94.8	94.8	94.8	94.8	94.8	94.8	94.8	
48 Hrs:	93.2	91.5	86.9	90.5	90.4	86.3	87.0	88.8±2.3

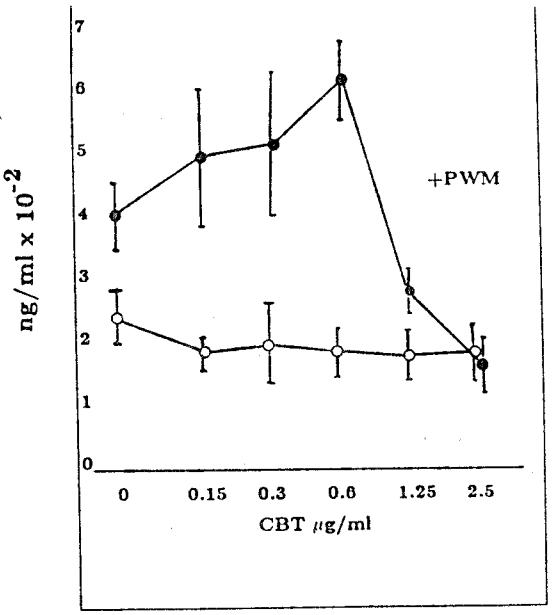

Fig. 1. Immunoglobulin G (ng/ml) in supernatants of 8 day cultures of 5 x 10^5 PBMC from a normal subject, containing CBT with (•) or without (o) PWM. With PBMC from two other subjects the PWM-induced IgG concentrations with 1.25μg/ml CBT, were 76% and 60% of their respective control values without CBT.

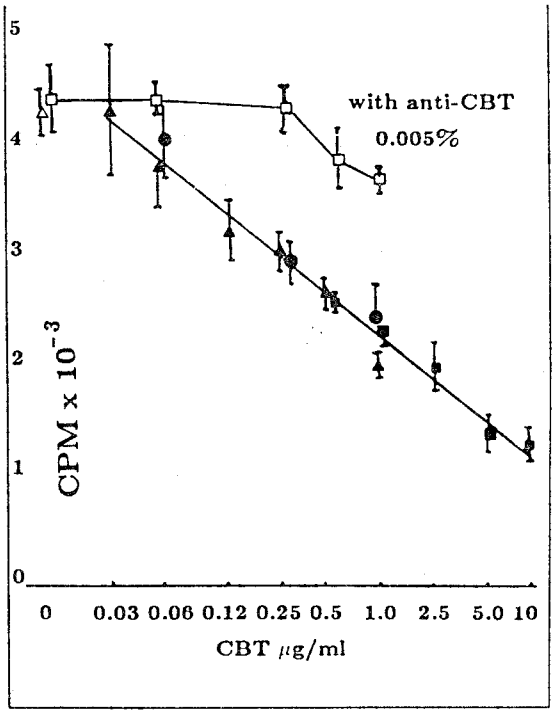

Fig. 2. Proliferative response of IL2-dependent NK cells in the presence of IL2, assayed in three separate experiments (▲, •, ■) with CBT. Growth of these cells in the presence of CBT and antiserum to CBT at 0.005% (□). Cells were cultured at 10^5/ml for 64 hours with a tritiated thymidine pulse for the last 16 hours.