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Impairment of T cell activation in burn patients: a possible mechanism of thermal injury-induced immunosuppression

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SUMMARY

In the burn patient, the mechanisms leading to impaired T lymphocyte activity are unclear. The capacity for T cell proliferation and the expression of Tac antigen (IL-2 receptor) was assessed during the post-burn period in patients with injuries ranging from 5–68% total body surface area. T cell-dependent (polyclonal) immunoglobulin synthesis, mixed lymphocyte reaction and Interleukin-2 production were also determined in these patients and correlated with survival. Surviving patients demonstrated a transient reduction while terminal patients exhibited a permanent reduction in the number of Tac (+) lymphocytes, unrelated to the absolute number of T cells, during the post-burn period. The reduced percentage of IL-2 receptor-expressing T cells coincided with the suppressed antibody response and reduced alloreactivity. Although the concentration of IL-2 was decreased in all patients throughout the hospitalization period, surviving patients showed a gradual increase in its production while terminal patients gradually decreased to undetectable levels. Exogenous recombinant IL-2 induced a significant enhancement of in-vitro polyclonal immunoglobulin production and blastogenesis in the mixed lymphocyte reaction in immunosuppressed patients who demonstrated up to 50% reduction in the percentage of IL-2 receptor positive cells. Thus, the reduced capacity for production of and response to IL-2 after thermal injury may lead to the immunosuppression due to a lack of T lymphocyte clonal expansion. The permanent nature of this defect in patients who died from fatal sepsis may suggest a causative relationship.

Keywords burn patients immunosuppression IL-2 receptor Tac antigens

INTRODUCTION

It has been suggested that the impaired regulation of T lymphocyte activity in thermally injured patients is due primarily to the development of suppressor T cell subsets during the post-burn period (Antonacci *et al.*, 1984; Munster, 1976; Ninneman & Stein, 1980; Warden & Ninneman, 1981; Winkelstein, 1984). Additionally, endogenous soluble factors with immunosuppressive activity have been demonstrated in sera from burn patients (Constantian, 1978; Ozkan & Ninneman, 1985).

The most fundamental defect resulting from the burn injury may involve dysfunction of helper T lymphocytes. Recent data from this laboratory show that T cell-dependent polyclonal immunoglo-

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bulin synthesis in pokeweed mitogen (PWM)-activated cultures of burn patient's lymphocytes was intermittently or permanently suppressed in parallel with alterations in alloreactivity as measured in the mixed lymphocyte reaction (MLR) against selected stimulator cells (Teodorczyk-Injeyan *et al.*, 1986). These findings indirectly suggested quantitative and/or functional changes within the helper T cell subset as a consequence of thermal injury (Hirano *et al.*, 1977; Keightly, Cooper & Lawton, 1976; Kondo, Orii & Uetake, 1983). Indeed, phenotypic data have demonstrated that the percentage of OKT4 helper/inducer cells decrease following the burn injury and trauma (Antonacci, Good & Gupta, 1982; O'Mahony *et al.*, 1984). The production of T cell growth factor (IL-2), produced mostly by T helper cells (Pfizenmaier *et al.*, 1984), was found to be significantly depressed in burn patients (Antonacci *et al.*, 1984; Wood *et al.*, 1984). However, the deficiency in IL-2 production was sustained in these patients even after the number of OKT4 lymphocytes returned to the normal level (Wood *et al.*, 1984). Recent results (Antonacci *et al.*, 1984) showed that the autologous MLR, studied in patients at 24–48 h post-burn, could be augmented by exogenous IL-2. As these observations may provide a guideline for a specific immunomodulation of the burn patient's immune response, elucidating cellular and molecular mechanisms for the decreased T cell function post-burn remains particularly relevant.

IL-2 promotes the proliferation of any T lymphocytes endowed with specific IL-2 membrane receptors, regardless of their subset and antigenic specificity (Schreier *et al.*, 1980). The rate of T cell proliferation is directly dependent on the concentration of IL-2 available to the cells (Smith, 1983), on IL-2 receptor density, and on the duration of the IL-2 receptor interaction (Cantrell & Smith, 1984).

This paper reports alterations in expression of the IL-2 receptor (Tac antigen), and of IL-2 production in surviving and non-surviving burn patients. These were profoundly decreased during the post-burn period while T cell numbers remained relatively constant. The surviving patients' capacity for restoration of the immune response in the presence of exogenous IL-2 appeared to depend on the level of IL-2 receptor-bearing lymphocytes.

MATERIALS AND METHODS

Patients. Patients ranged from 28–85 years and had injuries representing 5–68% total body surface area (TBSA)

Therapy. At the time of admission patients were resuscitated using a modified Parkland formula (Curreri, Richmond & Marvin, 1974). Enteral feedings were begun within 72 h of admission. Burn wounds were treated by twice daily tubbings (or showering) and by the topical application of silver sulfadiazine ointment (Flamazine, Smith & Nephew Inc., Lachine, Quebec). Prophylactic antibiotics were not administered.

Preparation of peripheral blood mononuclear cells (PBM). To avoid possible variables due to the method of selection or the loss of cells during the preparative process, whole blood mononuclear cell preparations were studied. Heparinized blood samples were collected within 24 h of the injury (with one exception) and subsequently at 10 day intervals. PBM were isolated by gradient centrifugation on Ficoll-Hypaque, washed in RPMI 1640, and resuspended in a complete tissue culture medium (TCM) consisting of RPMI 1640 supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine and antibiotics. In activation studies, TCM was further supplemented with 5×10^{-5} M 2-mercaptoethanol.

Immunoglobulin production and assessment. Culture conditions for PWM-induced polyclonal immunoglobulin synthesis and determination of levels of IgG and IgM in the culture sypernatants by enzyme-linked immunosorbant assay (ELISA) were performed as described (Teodorczyk-Injeyan *et al.*, 1986). Supernatants from replicate cultures (three) were collected, stored at $-70°C$, and then simultaneously assayed against internal standards (aliquots of TC supernatant of cultures prepared from normal donors).

Mixed lymphocyte reaction. Patient's PBM (5×10^5/ml) were co-cultured with mitomycin C-treated human EBV-transformed lymphoblastoid cells (LC) prepared as described by Nilsson (1976). Two lines of LC of determined phenotypes were initially tested for each patient and the optimal ratio of responder to stimulator cells was established during the first test. The incorporation

of [^{3}H] Tdr was measured on day 6, after a 16 h pulse. The first test established MLR responsiveness presumably due to differences at the class II locii.

Activation assay and enumeration of Tac (+) cells. PBM were incubated with phytohaemagglutinin (PHA) (10 μg/ml) or concanavalin A (Con A) (10 μg/ml) for 72 h in TCM. Cells were then washed thoroughly in PBS and incubated with a monoclonal anti-IL-2 receptor phycoerythrin-conjugated antibody (100 ng/10^6 cells, Becton/Dickinson). Cells were then washed, resuspended in 1% paraformaldehyde in PBS and analysed on an EPICS V Flow Cytometer (Coulter Electronics, Hialeah, FL). The percentage of Tac (+) cells (IL-2 receptor-bearing) (Miyawaki *et al.*, 1982; Robb, Greene & Rusk, 1984) was estimated by enumeration of the stained cells considering appropriate controls of non-specific labelling with normal mouse IgG_1 and staining of unstimulated (incubated without mitogen) patients' lymphocytes.

Monitoring T cell levels. PBM were washed in PBS and stained with monoclonal anti-Leu-1 (human T cell antigen) fluorescein-conjugated antibody (Beckton/Dickinson) at 5 μl/10^6 cells. The number of stained cells was determined by flow cytometry as described above for enumeration of Tac (+) cells.

Determination of IL-2 secretion. The supernatants of PHA-stimulated (10 μg/ml, 48 h) PBM were assessed for IL-2 activity using the murine IL-2-dependent 2.8.2 cell line kindly provided by Drs C. Havele and V. Paetkau. One unit of activity was defined as the reciprocal dilution required to give 1/3 maximal proliferation of 10^4 cells/ml after 24 h culture (Bleackley, Havele & Paetkau, 1982).

Exogenous IL-2. IL-2 derived from cDNA for human IL-2 expressed in *E. coli* (recombinant IL-2) was a gift from Biogen Research Corp. (Cambridge, MA) (Devos *et al.*, 1983). Following reconstitution the recombinant IL-2 was assayed on the 2.8.2. cell line and was used throughout this study at a concentration giving maximal stimulation (20 U/ml) (Roifman *et al.*, 1985).

Control values. The IL-2 production and expression of Tac antigen were determined in six healthy volunteers 29–45 years of age.

Statistics. The results are expressed as means of values per assay ±s.d. The statistical significance of the differences was determined by Student's *t*-test. A value of $P < 0.05$ was considered significant.

RESULTS

Patients. Five of ten patients presented in this study developed sepsis and died. Two of these were elderly, the others had large burns (TBSA > 60%) with full thickness injuries ranging from 28–65% (Table 1). Nine of the patients under study had an average of four successive tests performed

Table 1. Patient data (patients 1–5 survived, patients 6–10 died during the study)

		Associated injury			
Patient	Age	TBSA	Full (%)	Partial	Remarks
1	36	45	40	5	
2	39	35	29	6	
3	50	45	35	10	
4	28	5	5	0	Electrical injury
5	32	55	45	10	
6	72	31	30	1	
7	43	68	65	3	
8	46	65	63	2	
9	85	28	28	0	
10	40	45	45	0	Diabetes

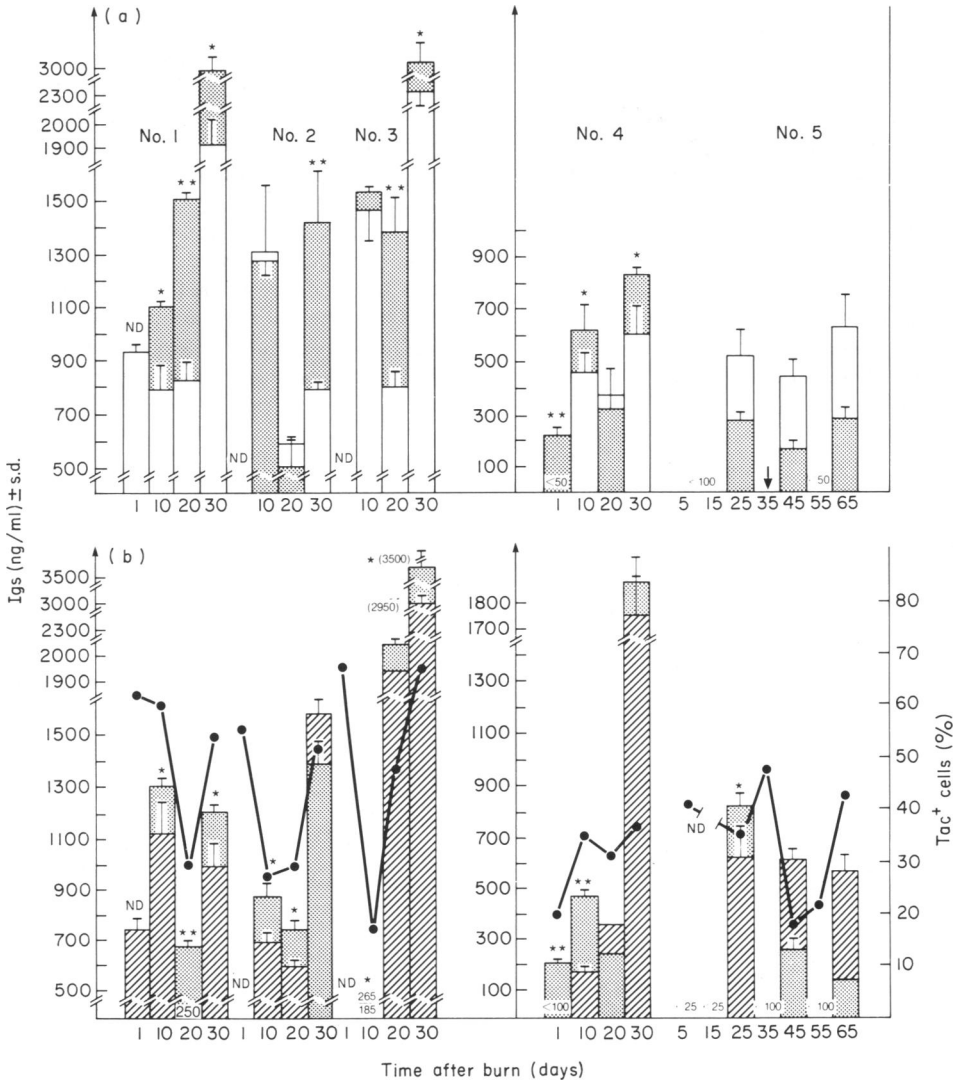

Fig. 1. Effect of exogenous IL-2 (20 U/ml) on pokeweed mitogen-induced immunoglobulin production in PBM cultures from surviving burn patients. Cultures of 1×10^6 PBM were established on days shown after burn. The levels of (a) IgG (□) and (b) IgM (■) were determined as described in Materials and Methods, after 7 days of incubation in intact and IL-2 supplemented (a & b, ▨) cultures. Columns illustrate the mean quantities (± s.d.) of Ig in the supernatant from triplicate cultures. Figures are used instead of columns when the levels of Ig in the tested supernatants did not exceed 100 ng per ml. The percentage of Tac (+) cells (●) in these patients was determined by direct immunofluorescence, in parallel ConA-activated cultures after 72 h incubation (see Materials and Methods), and was superimposed on the respective results illustrating Ig synthesis. * $P < 0{\cdot}05$, ** $P < 0{\cdot}001$ ND = not determined, ↓ = not detected.

(range 3–7). Patient No. 10 died of complications from septicemia and pneumonia 5 days after his second test.

Polyclonal immunoglobulin (Ig) synthesis and MLR. Our preliminary studies in normal volunteers of different ages ($n=20$) established that while inter-donor variability in polyclonal Ig production and the MLR was wide, intradonor variability in 3 to 5 tests performed within 30–60 days was not significant ($P<0{\cdot}23$). We have also observed that the patient's first day and discharge time responses were not different from those found in healthy donors and they also demonstrated a similar range of variability (Teodorczyk-Injeyan *et al.*, 1986). Thus these responses were adopted as the patients' self-control. Generally cultures from all patients responded to PWM stimulation by producing greater than background quantities of IgG and IgM throughout the study: background (spontaneous) levels (determined in cultures incubated without PWM) were subtracted to give the depicted values (Figs 1 & 2). With the exception of one test (on patient No. 5 and 35 days after injury), the background production of IgG never exceeded that found in PWM-activated cultures. All patients demonstrated one or more periods of suppressed antibody response (Figs 1 & 2) relative to both normal controls (range: 290–1877 ng/ml IgG, 400–3623 ng/ml IgM) and to their baseline level, determined at the time of admission and/or discharge. The duration and degree varied but it was usually apparent 2–3 weeks after the injury and involved at least one class of Ig. However, the

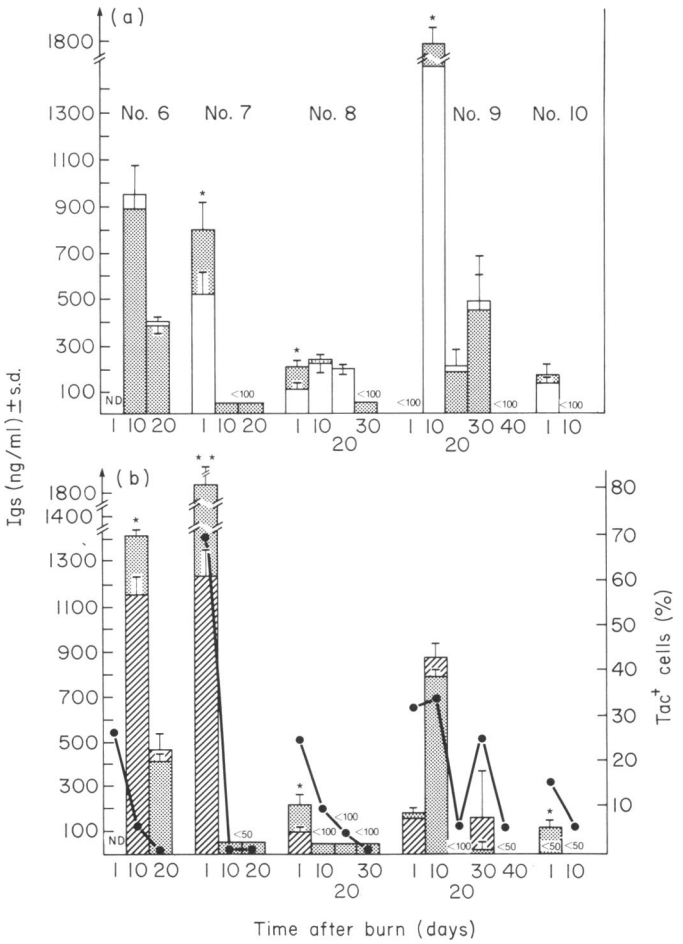

Fig. 2. Effect of exogenous IL-2 (20 U/ml) on pokeweed-mitogen induced immunoglobulin production in PBM cultures from non-surviving burn patients. Details as Fig. 1.

pattern of alterations in the humoral response was different in the surviving and non-surviving patients.

Survivors (patients No. 1, 2 and 3, Fig. 1) demonstrated an intermittent suppression of Ig production manifested by significantly reduced ($P < 0{\cdot}05$–$0{\cdot}001$) levels of IgG and/or IgM between 10 and 30 days post-burn. The secretion of IgG, in PWM-activated cultures, was reduced by 80 to > 800 ng/ml, and of IgM by 140 to > 400 ng/ml, (in patients No. 1, 2 and 3) in comparison with the initial levels demonstrated before 10 days post-burn. The antibody production in patients No. 4 and 5 was strongly suppressed during the initial phase of the monitoring period (less than 100 ng/ml of Ig in their first test). At the time of discharge both patients' Ig synthesis was restored to levels significantly higher ($P < 0{\cdot}001$) than those observed at the time of admission and not significantly different from normal controls (IgG > 600 ng/ml in both patients; IgM: 1745 and 667 ng/ml, respectively). At that time the immunoglobulin production in the remaining patients (no. 1, 2, and 3) was also significantly higher ($P < 0{\cdot}05$– $< 0{\cdot}001$) than that demonstrated during the mid-phase of the post-burn period.

Within the first 10 days of the post-burn period PWM-induced Ig synthesis in non-surviving patients (Fig. 2) was not different from those demonstrated by the surviving patients and ranged from a low (100–300 ng/ml in patients No. 8 and 10) to normal (IgG: 520–1420 ng/ml, IgM: 800–1250 ng/ml) level. However, the subsequent tests showed that polyclonal antibody responses became progressively and irreversibly suppressed in all but one (No. 6) of these patients, to less than 100 ng/ml, for 3 to > 20 days before death.

We have previously demonstrated that in burn patients the alterations in the magnitude of the Ig synthesis, and the MLR, as measured by incorporation of [^{3}H]TdR in standardized stimulator-activated cultures, show a good to excellent degree of correlation (Teodorczyk-Injeyan *et al.*, 1986). Changes in alloreactivity in patients presented in this study also followed the pattern of the polyclonal antibody response. The initial (day + 1) response of surviving patients (Fig. 3) was not

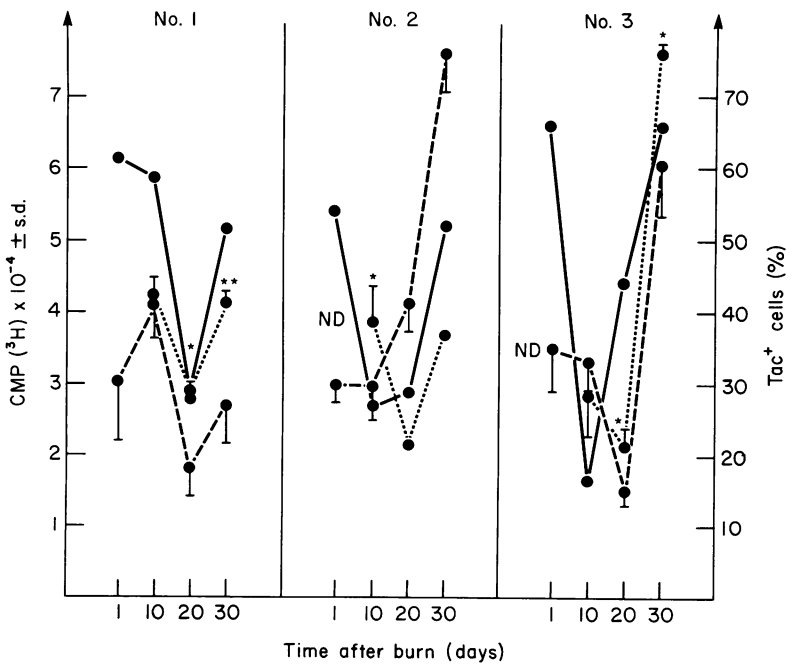

Fig. 3. Effect of exogenous IL-2 (20 U/ml) on the mixed lymphocyte reaction in surviving burn patients. The results represent the mean (± s.d.) [^{3}H]-thymidine incorporation into patients PBM (5×10^4 cells, quadruplicate cultures) cultivated with mitomycin-treated LC at a predetermined ratio (see Materials and Methods). The cultures were established on different days after burn and maintained for 6 days with (● ..... ●) and without (● - - - - ●) IL-2. The percentage of Tac (+) (●—●) was determined as described in the legend to Fig. 1.

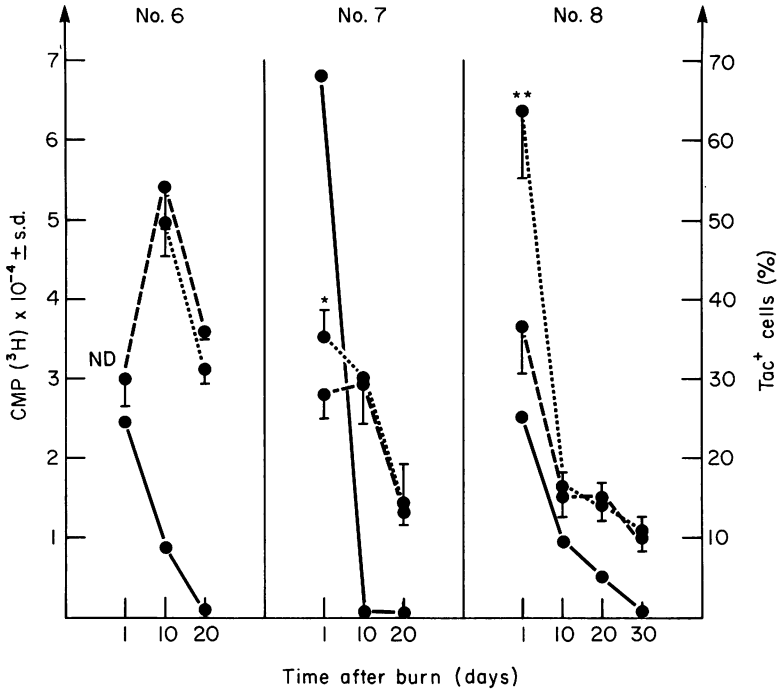

Fig. 4. Effect of exogenous IL-2 (20 U/ml) on the mixed lymphocyte reaction in non-surviving burn patients. Details as in Fig. 3.

different from that of those who did not survive (Fig. 4) and showed a narrow range of proliferation at 27,850–37,011 cpm/culture. The surviving patients, then, demonstrated a significant ($P < 0{\cdot}05$) but transient decline in cell proliferation (by 40–58%, patients No. 1 and 3) followed by the restoration of blastogenesis to its original (patient No. 1) or significantly higher ($P < 0{\cdot}001$, patients No. 2 and No. 3) levels (over 60,000 ct/min/culture) at the time of discharge. In contrast, non-surviving patients demonstrated a permanent decrease of blastogenic responses (by 37–66%) apparent shortly or several weeks before death (Fig. 4).

Circulating T lymphocytes. The profound decrease in the response to polyclonal mitogen and in the MLR, may have resulted from a non-specific decrease in total T cell number. When we tested this by immunofluorescence with the pan T cell marker Leu-1 the decrease in percentages of circulating T cells was usually less than 30% (see below). This decrease, unlike the profound decrease in mitogen and MLR responsiveness, suggested we examine the response to and the production of IL-2, the lymphokine which appears to regulate the magnitude and the extent of many immune responses.

The effect of exogenous IL-2. PWM-activated and LC-stimulated cultures were supplemented with recombinant IL-2 (20 U/ml) at the time of their initiation. In four patients (Nos. 4, 5, 8 & 9) additional cultures were supplemented with IL-2 after 72 h of incubation, with no effect.

Cultures from surviving patients demonstrated a significant and frequent increase ($P < 0{\cdot}05$–$P < 0{\cdot}001$) in the production of IgG (9/20 tests) and IgM (10/20) in the presence of IL-2 (Figs 1 a,b); they responded to IL-2 treatment by increasing the synthesis of IgG by 170–1080 ng/ml and of IgM by 198–550 ng/ml. In particular, patient No. 1 demonstrated, in three consecutive tests with IL-2, an increase in the IgG and IgM production by 310–1080 and 198–330 ng/ml, respectively. Patients No. 2 and No. 3 showed a response to IL-2 by increasing mainly the IgG (by 80–750 ng/ml) in three tests including the period of suppressed antibody production. Patient No. 4 showed a significant increase ($P < 0{\cdot}001$) in the production of Ig in two consecutive tests, and antibody production in the presence

of IL-2 in this patient was restored to the levels approaching those in normal donors (Fig. 1 a,b).

In contrast, cells from patients who did not survive had a lower and less frequent IgG and IgM response to IL-2 (3/16 and 4/16 tests, respectively), and these were restricted to the first test (Fig. 2 a,b). IL-2 did not augment their responses subsequently. For example an increase in the IgG production (by 103–290 ng/ml) was observed in three non-surviving patients (nos. 7, 8 and 9) in their initial tests, and the synthesis of IgM was similarly augmented (by 100–260 ng/ml) in patients No. 6, 8 and 10, and by 585 ng/ml in patient No. 7, at the time of admission.

In the MLR, a response to exogenous recombinant IL-2 was apparent in the surviving group of patients, with a significant augmentation of the proliferative response in five out of nine of the tested cultures (Fig. 3). The addition of IL-2 to these cultures significantly increased ($P < 0{\cdot}05$–$P < 0{\cdot}001$) [^{3}H]TdR incorporation in LC-stimulated cultures, both during the period of the suppressed MLR and at the time of patient discharge (patients No. 1 and No. 3). Cultures from non-surviving patients were generally unresponsive to exogenous IL-2 over the hospitalization period; a significant effect of this lymphokine ($P < 0{\cdot}05$ and $P < 0{\cdot}001$) was demonstrated in only two tests, and these were at the time of the patients' admission (Fig. 4). Occasionally IL-2 had a significant effect on the background proliferation of the PBM in the MLR, whether from survivors or non-survivors. The respective background proliferation of the responder cells was subtracted from the total values to give the presented values.

Production of IL-2. Generally, culture supernatants from cells obtained less than 24 h after the injury, and stimulated with PHA, contained measurable, but decreased, concentrations of IL-2 compared to normal control values. Figure 5 illustrates the changes in the IL-2 activity in six patients. The non-survivors (Nos 6, 7 & 8) developed total and permanent inhibition of IL-2 production, while survivors (Nos 1, 2 & 3) gradually restored their capacity to synthesize and/or secrete IL-2, though at a reduced level.

Expression of IL-2 receptors (Tac antigen). Expression of the IL-2 receptor was assessed by direct immunofluorescence using anti-IL-2 receptor antibodies. Preliminary experiments established that the numbers of Tac-positive cells in ConA or PHA-activated PBM from normal, age-matched donors, ranged from 23–71%. Considering this wide variability, the enumeration of Tac

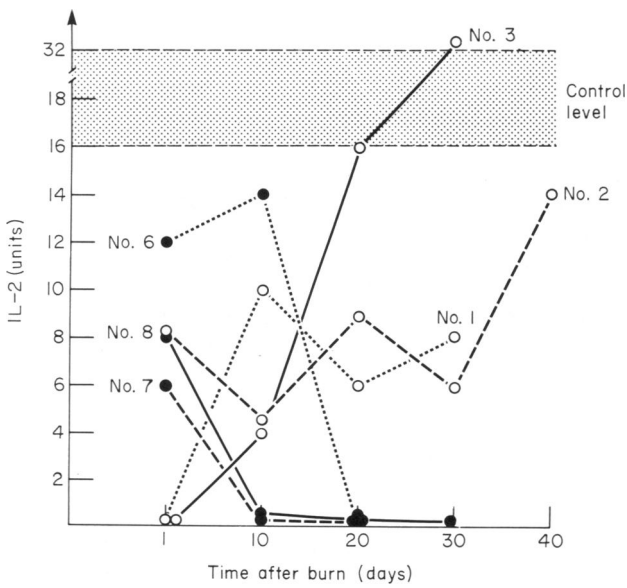

Fig. 5. PHA-induced IL-2 production in burn patients. PBM (1×10^6/ml) from the surviving (○) and non-surviving (●) burn patients were incubated for 48 h with PHA (10 μg/ml). Serial dilutions of each culture supernatant were then assayed on the IL-2-dependent 2.8.2 cell line (see Materials and Methods). One unit was defined as the reciprocal dilution required to give 1/3 maximal proliferation of 10^4 cells/ml in 18 h.

(+) lymphocytes on admission was utilized as a self-control. ConA-activated cells proved to provide an increased expression of Tac antigen in the burn patient and this mitogen was used in subsequent studies.

The number of cells expressing the Tac antigen varied considerably during the post-burn period in both groups of patients. Those who eventually survived demonstrated a higher (but not significant) percentage (48·6 ± 19%) of these cells than the non-survivors (34·6 ± 21%) at the time of admission. Over the monitoring period, survivors (except patient No. 4) showed a transient reduction in the number of IL-2 receptor bearing lymphocytes (Figs 1 & 3) while the non-survivors showed a progressive decrease in Tac (+) cells (Figs 2 and 4). For 1–2 weeks preceding the development of fatal sepsis, cultures with ConA did not augment expression of the Tac antigen. The changes seen in the patient's immune reactivity and the patient's response to exogenous IL-2 were related to the reduction in the number of IL-2 receptor expressing cells as measured by direct immunofluorescence (Figs 1–4). In the surviving patients, this reduction did not exceed 50% of the initial percentage of Tac (+) cells. The profound depletion of the IL-2 receptor-bearing cell population observed in non-survivors (as well as in one survivor, patient No. 3), was associated with non-responsiveness to exogenous IL-2.

Comparison between number of T lymphocytes and Tac (+) cells. The total number of Leu-1 (T) lymphocytes was examined periodically in all patients during the post-burn period. Compared to their initial baseline level, which was similar to that of normal donors (68% ± 12%) the numbers of Leu-1 cells were reduced by 5–30% at 10–30 days post-burn in both groups of patients. However, no significant correlation could be found between the absolute number of cells with Leu-1 phenotype, the magnitude of the immune responses, the IL-2 production and the percentage of Tac (+) determined at the same time ($P > 0{\cdot}5$). Thus, in patient No. 3 who demonstrated a profound decrease (by 72%) in the number of IL-2 receptor-expressing cells on day +10, the total number of Leu-1 cells had decreased only by 14% of the baseline number (from 67% to 58% of PBM) (Fig. 1), and returned in subsequent tests to 70·1%. Non-surviving patients No. 7 and No. 8, who showed 80% to >95% reduction in the number of IL-2 receptor-endowed cells, displayed a significant suppression of the immune response (Figs 2 & 4) from day +10 on, yet maintained Leu-1 cells at the level of 45%–50·5% of PBM throughout. The decreased responsiveness of patient's cells in each of these assays could not, therefore, be explained by a non-specific decrease in total T cell number.

DISCUSSION

Dysfunction of the T cell compartment of the immune response is a sequel to thermal injury (Antonacci *et al.*, 1984; Warden & Ninneman, 1981; Winkelstein, 1984). The cellular and T cell-dependent humoral responses of the patients presented in this study were suppressed or totally abrogated during the post-burn period. A reduced capacity for both IL-2 production and T cell activation was also demonstrated in these patients.

Normally, peripheral blood T cells undergo cell division when stimulated by alloantigens or PWM, and IL-2 provides the necessary signal in T cell activation by triggering the proliferation of IL-2 receptor-expressing lymphocytes (Larsson, 1981; Miyawaki *et al.*, 1982; Robb, Munck & Smith, 1981). A diminished proliferative response of T cells to mitogens and antigens in burn patients has been well documented (Miller & Baker, 1976; Xi-Ming *et al.*, 1983). This could result from a reduction in IL-2 production, from an inability of T cells to respond to IL-2, or from a combined defect.

Soluble mediators of the immune response can substitute for an absent or malfunctioning subset of T lymphocytes in cellular interactions (Gillis, 1983). The addition of exogenous IL-2 was therefore assayed *in vitro* during the post-burn period. IL-2 increased the antibody response and MLR in some patients, but seldom to their optimal (baseline) levels. Similar effects of exogenous IL-2 on autologous MLR were observed in burn patients studied 24–48 h post burn (Antonacci *et al.*, 1984). This suggested that patients may differ in the capacity of their T lymphocyte population to respond to available IL-2. Thus, a defect in the production of IL-2 in burn patients is not the only burn injury-induced failure of T cell function.

The number of cells expressing IL-2 receptors following activation by mitogens in patients

under study was greatly reduced either temporarily or permanently. The profound or total failure of Ig synthesis and MLR-induced proliferation was associated with a decrease in IL-2 receptor expression and a lack of response to exogenous IL-2. In contrast, patients who were responsive to IL-2 *in vitro*, demonstrated only a moderate and transient reduction in IL-2 receptor expression, followed by a relatively fast recovery to initial (baseline) levels of Tac (+) cells and of immune reactivity.

A significant reduction in numbers of T lymphocytes, particularly of the helper T cell subset, is usually observed after burn injury (Antonacci, Good & Gupta, 1982; O'Mahony *et al.*, 1984; Wood *et al.*, 1978). The most evident lymphopenia is observed up to 5 days after the injury (Munster *et al.*, 1980; Neilan, Taddeini & Strate, 1977). However, diminished production of IL-2 is apparent considerably longer than the reduction in the number of OKT3 and OKT4 lymphocytes (Wood *et al.*, 1984). Quantitative fluctuations in T cell populations, observed in our patients, did not correlate with functional activity of these cells. It has now been established that there is no absolute correlation between the surface phenotype and the functional potential of human peripheral blood T cells (Moretta, 1985).

IL-2 is required for the optimal expression of the Tac antigen (Mills *et al.*, 1985; Reem & Yeh, 1984; Smith & Cantrell, 1985). Thus, the decreased production of IL-2 in burn patients might be responsible for a failure of T cells to generate and/or maintain sufficient densities of IL-2 receptors. Our recent studies on expression of IL-2 receptors in immunosuppressed burn patients show that the profoundly reduced percentage of Tac (+) cells can be restored to baseline levels in the presence of sufficient concentrations of exogenous IL-2 (unpublished results). Additionally, expression of IL-2 receptors is a transient phenomenon ultimately determined by antigen/lectin stimulation (Cantrell & Smith, 1983). Thus, a possibility that an inadequate activating signal is being delivered to a T cell must also be considered.

The IL-2 receptor, like some other growth factor receptors, appears to be expressed in both a high affinity and a low affinity form. Since anti-Tac antibodies bind to both forms of the receptor and the high affinity receptor appears to be the functional IL-2 receptor, anti-Tac antibody binding does not correlate directly with the expression of functional IL-2 receptors (Robb, Greene & Rusk, 1984; Smith & Cantrell, 1985). The present study assayed total receptor expression by direct immunofluorescence and functional receptor expression by IL-2-augmented proliferation. In the burn patient, these two assays showed a close correlation suggesting that the total number of receptors expressed paralleled the expression of the functional IL-2 receptor.

Eicosanoid metabolic pathways have been shown to be activated in burn patients (Ninneman, Stockland & Condio, 1983). Prostaglandins reduce expression of I-region associated (Ia) antigens on macrophages (Snyder, Beller & Unanue, 1982). Thus, insufficient antigen presentation to specific T cells, due to a modulated expression of Ia-like (HLA-DR) molecules on antigen presenting cells, could be operative in burn patients. The production of IL-1 in burn patients appears to be unaffected suggesting that in the presence of antigen or mitogen a second activating signal is delivered to T lymphocytes (Pfizenmaier *et al.*, 1984). The release of prostaglandins E_2 (PGE_2) in the burn patient may have caused activation of suppressor cells and inhibition of the production of IL-2 as observed for normal lymphocytes (Snyder, Beller & Unanue, 1982; Chouaib *et al.*, 1984). On the other hand the reduced IL-2 concentrations in supernatants of mitogen-activated T cells may have been mediated either by decreased production or by absorption of IL-2 by surface receptors on these cells (Palacios & Moller, 1981; Gunther, Haas & Von Boehmer, 1982). As demonstrated in this report the numbers of IL-2 receptor-expressing cells in burn patients were consistently and significantly reduced at the time of the suppressed immune reactivity.

The impaired expression of IL-2 receptors, decreased IL-2 production and low proliferative response to mitogens and alloantigens is similar to that seen in human thymocytes and in post bone marrow transplant patients (Roifman *et al.*, 1986). This raises the question whether the T cells present in the circulation of burn patients at this time, are immature and recapitulating ontogeny.

The findings presented in this study suggest that the burn injury affects the intrinsic regulatory mechanisms for the synthesis and/or release of IL-2 with the subsequent capacity for expression of IL-2 receptors decreased. This may possibly result from an inability to respond to an activating signal, and/or inadequate concentrations of available IL-2. Therefore, acquisition of IL-2 receptors

and their maintainance at maximum levels *in vivo* appears to be affected following the thermal injury. The resultant loss of responsiveness may then be due to the impaired T cell activation and clonal expansion.

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