Immunosuppression Following Excision of Burn Eschar and Syngeneic Grafting in Major Thermal Trauma

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Recent reports have suggested that very early excision (<24 hours post-burn) and primary closure of burn wounds might circumvent the immunosuppression which follows severe thermal trauma. The total body surface area (TBSA) involved in burn injuries of human subjects at risk for significant post-burn immunosuppression is large enough to require grafting. In the present study cell-mediated immunity was measured via one-way allogeneic mixed lymphocyte reactions (MLR) in mice subjected to full-thickness scald wounds over 25-30 percent TBSA followed by escharectomy and syngeneic full-thickness skin grafting. A significant decrease in the proliferative capability of T-cells could be demonstrated on days five and seven post-treatment in unburned grafted animals (day five, 30.7 percent; day seven, 24.8 percent) over untreated normals. T-cells from animals burned but not excised also showed significant hyporesponsiveness (day five, 33.2 percent; day seven, 26.1 percent normal MLR). Animals undergoing both burning and excision showed even more profound immunosuppression (day five, 18.3 percent to 23.7 percent; day seven, 7.4 percent to 11.6 percent normal MLR). Surgical incision without excising the skin did not suppress cell-mediated immunity (day five, 90.8 percent; day seven, 90.4 percent normal MLR). When T-cells from treated animals of each group (with the exception of the incision control group) were added to normal MLR cultures, significant (>50 percent) cell-mediated suppression by suppressor T-cells could be demonstrated. This study showed that the trauma of excision and grafting alone results in depression of cell-mediated immunity. These data call into question the ability of very early excision and grafting to alter the immunosuppression which follows severe thermal trauma.

INTRODUCTION

Formerly, major thermal trauma resulted in very early death due to respiratory or hemodynamic shock. With the advent of modern concepts of electrolyte and fluid replacement and the refinement of respiratory care techniques, most burn patients now survive the initial physiologic insult. A recent consensus report shows that currently greater than 75 percent of all post-burn deaths have infection as the primary contributing cause [1]. Investigations of both burn patients and murine models have demonstrated the induction of an active, nonspecific, T-cell-mediated immunosuppression following significant burn injuries [2,3]. These studies correlated the risk of sepsis with the appearance of suppressor cell activity in a T-cell-enriched cell suspension in vitro. More recently Kupper and Green [4] have examined the physiologic significance of this suppression phenomenon in vivo. By injecting T-cells from seven day post-burn (immunosuppressed) mice into syngeneic animals that were subse-
Anesthesia
Animals
via mice and Harbor, Laboratories), using the tribute have expression thickness skin community can't cell-mediated early chemotaxis quantitative in reticuloendothelial system [9,10], and alterations in neutrophil function and chemotaxis [11,12], the action of the T-suppressor cell circuit on the humoral and cell-mediated immunity of the burned host is sufficient to put the subject at significant risk for a fatal septic episode. Therefore, in these experiments cell-mediated immunity was used as an index to compare the effects of untreated burn-induced immunosuppression with the immunocompetence of mice burned and followed by very early excision (<24 hours post-burn) of the burn wound and syngeneic full-thickness skin grafting.

In an investigation of the immunocompetence of severely burned human patients, Miller and Baker discovered that the degree and duration of post-burn immunosuppression was related to the extent of the burn injury [2]. Several other investigators have directly isolated factors from the burn eschar or characterized local reactions to the tissue trauma that have immunosuppressive activity [13,14,15,16]. All of the above observations imply that some factor or factors from the burned tissue contribute to the induction of the post-burn immunosuppression. Therefore, removal of the eschar before such factors could be released systemically could conceivably avert the suppression by eliminating the inciting agents.

Using a standard murine model consisting of a 25–30 percent TBSA full-thickness scald wound, this work studied the effect of very early escharectomy on the ultimate pathology of cell-mediated post-burn immunosuppression. In the human burn patient at significant risk for a fatal sepsis resulting from suppressed immunity as a complication of the trauma, the burned area is much too large to allow for a primary closure following escharectomy [2]. Therefore, to approximate the clinical situation in burn patients the open escharectomy wound in our murine model was covered with a full-thickness skin graft from a syngeneic mouse. Both temporal and quantitative studies were conducted to investigate (1) the potential benefits of very early excision of burn eschar, (2) the effects on cell-mediated immunity of skin grafting with syngeneic full-thickness skin flaps following very early post-burn escharectomy, and (3) the effects of the surgical trauma of syngeneic grafting alone on cell-mediated immunity by subjecting unburned mice to skin excisions and grafting.

**MATERIALS AND METHODS**

*Animals*

Male CBA/J and C57Bl/6 mice were obtained from Jackson Laboratories, Bar Harbor, Maine, and were six to ten weeks old when treated. Following treatment the mice were kept four to six per cage and allowed food and water ad libitum.

*Anesthesia*

All mice were anesthetized with an intraperitoneal injection of Nembutal (Abbott Laboratories), using 1 mg/22 g body weight. Ether supplementation was provided via a nose cone, as needed.

*Burn Injury*

The dorsum of each anesthetized mouse was shaved with an Oster animal clipper and an area corresponding to approximately 30 percent of the total body surface
area (TBSA) was outlined with a marking pen. This area was calculated relative to the animal's body weight as described by Schildt and Nilson [17]. The mouse burn template used was a seven-inch-long, hollow, open-ended Teflon cylinder (diameter = 2.2 cm) with a rectangular opening 2 × 4.5 cm midway on its length through which the area to be burned could be exposed. A full-thickness scald wound was inflicted by dipping the exposed skin into 95°C water for eight seconds. The mouse was then immediately removed from the template and patted dry with a towel. Fluid resuscitation was administered in the form of one ml of injectable 0.9 percent saline (Invenex) injected intraperitoneally immediately post-burn and again six hours post-burn.

**Escharectomy and Skin Grafting**

At the appropriate times post-burn the mice were again anesthetized with intraperitoneal injections of Nembutal. The burn eschar was then removed from the underlying tissue by careful dissection. The burn model described above facilitated this procedure by consistently producing uniform full-thickness (third-degree) burns with sharp margins. Full-thickness syngeneic skin grafts were obtained from freshly sacrificed mice of the same strain (i.e., CBA/J or C57Bl/6) using previously described procedures [18]. Briefly, the animals were sacrificed by cervical dislocation, their dorsums shaved, and an area corresponding to 30 percent TBSA demarcated and removed from the underlying tissue by careful dissection. The graft was secured to the previously burned mouse with 3-0 dermalon sutures (Davis and Geck) using a continuous horizontal mattress stitch. Unburned excision control animals were similarly prepared by exchanging full-thickness skin flaps of about 30 percent TBSA between two previously untreated (i.e., unburned) syngeneic mice. In all surgical cases fluid resuscitation again consisted of one ml of 0.9 percent saline injected intraperitoneally immediately post-surgery and again six hours post-surgery.

**Incision Controls**

Nembutal-anesthetized mice were shaved and marked as above. An incision was made in the skin along the demarcation around the dorsum of the mouse but the skin was not dissected away from the underlying tissue. The incision was closed and the animals resuscitated as previously described.

**Spleen Cell Recovery**

On the appropriate days post-treatment the mice were sacrificed by cervical dislocation along with several untreated syngeneic mice (normal controls) and several untreated allogeneic mice (stimulator pool). The spleens were removed aseptically and ground between the frosted ends of two glass slides into Cantor's balanced salt solution (Irvine Scientific) and pooled appropriately. Red blood cells were removed by hypotonic shock.

**T-Cell Enrichment**

T lymphocytes were fractionated on the basis of surface antigens as described by Wysocki and Sato [19]. In brief, goat anti-mouse immunoglobulin (obtained from Dr. M. Iverson) was adsorbed on to sterile polystyrene Fisher petri dishes and the remainder of the nonspecific Fc binding sites saturated with goat IgG. The plates were stored at −4°C until needed. 3 × 10⁷ spleen cells in 5 ml of balanced salt solution were added to each plate and allowed to stand at +4°C for 60 minutes. The nonadherent cells (T-cells) were then carefully decanted with a total cell recovery of 30–45 percent.
**Mitomycin C Treatment**

Stimulator cells were prepared by treating whole spleen cell suspensions with 40 μg/ml of Mitomycin C (Sigma) at concentrations of $2.5 \times 10^7$ cells/ml in a balanced salt solution. After having been incubated for 20 minutes at $30^\circ C$ the cells were washed three times in 30 ml of balanced salt solution to remove all traces of Mitomycin C.

**Cell Cultures**

Viable cells were counted by the Trypan Blue (Gibco) exclusion method and diluted appropriately in Click’s medium (supplied by Dr. M. Iverson) supplemented with 5 percent fetal calf serum (Gibco) and 1 percent penicillin-streptomycin (Irvine Scientific). Stimulators and responders were chosen such that they differed at the H-2 or major histocompatibility locus (i.e., C57Bl/6 × CBA/J). Cultures were arranged as indicated in Fig. 1, in triplicate, in Costar microtiter culture plates with a final volume of 0.2 ml. The cultures were then incubated at $37^\circ C$ in 95 percent air/5 percent CO$_2$ for 96 hours. At this time each well was pulsed with 1.5 μCi of $^3$H thymidine (New England Nuclear) and incubated for an additional four to six hours. Cultures were then harvested, using a MASH, on to Whatman glass microfiber filter paper (Whatman 934-AH). After complete drying, the individual discs of filter paper were mixed with 1 ml of Betafluor (Beckman) in glass scintillation vials and the counts measured on a Beckman liquid scintillation counter for one minute.

**Statistics**

Spleen cells from four to six treated or control animals were appropriately pooled for each experiment and the lymphocyte cultures arranged in triplicate. Therefore, for each experimental condition reported $n$ was a multiple of three and the reported $n$ reflects the number of times the experiment was repeated. The results were compared using a standard two-tailed student’s $t$-test with statistical significance defined as a $p < 0.05$.

| Experimental Cultures | $S^* \times N$ | $S^* \times 2N$ | $S^* \times 3N$ | $S^* \times R5d$ | $S^* \times R7d$ | $S^* \times N \times R5d$ | $S^* \times N \times R7d$ |
|-----------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| S*                    | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ |
| N                     | $2 \times 10^4$ | $4 \times 10^4$ | $6 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ |
| R5d                   | $4 \times 10^4$ | $4 \times 10^4$ | $4 \times 10^4$ | $4 \times 10^4$ | $4 \times 10^4$ | $4 \times 10^4$ | $4 \times 10^4$ |
| R7d                   | $4 \times 10^4$ | $4 \times 10^4$ | $4 \times 10^4$ | $4 \times 10^4$ | $4 \times 10^4$ | $4 \times 10^4$ | $4 \times 10^4$ |

| Control Cultures      | $R^* \times N$ | $R^* \times 2N$ | $R^* \times 3N$ |
|-----------------------|----------------|----------------|----------------|
| R*                    | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ |
| N                     | $2 \times 10^4$ | $4 \times 10^4$ | $6 \times 10^4$ |

**FIG. 1.** Experimental design of Mixed Lymphocyte Reactions—cells per culture: all cultures in total volume of 0.2 ml; $S^*$ mitomycin C-treated stimulator cells; $N$ responder cells from normal (untreated) mice; $R$ responder cells from appropriately treated mice sacrificed on post-burn day indicated; $R^*$ mitomycin C-treated syngeneic (to N) cells.
RESULTS

Mortality

The insult of a full-thickness scald wound over 25-30 percent TBSA led to a 30 percent mortality of which three-fourths occurred in the first 48 hours and were attributable to the physiologic shock produced by the burn injury. The remainder were due to sepsis (Fig. 2). Mice burned and then excised and grafted at six and 24 hours experienced mortality rates of 50 percent and 40 percent, respectively. Again, roughly three-fourths could be attributed to shock and the other fourth to sepsis. Those mice excised and grafted without the antecedent burn suffered a 10 percent mortality (all septic episodes) while those in the incision control group all survived.

Immunosuppression Following Wound and Grafting

At six or 24 hours post-burn, groups of mice underwent escharectomy and syngeneic grafting as previously described. By day five following the escharectomy, the avascular skin graft was adherent to the back of the recipient mouse and grossly dehydrated. Careful dissection of the avascular graft from its recipient revealed an abundant granulation response on the graft bed. On days three, five, and seven post-burn animals were sacrificed and their cell-mediated immunity measured via a one-way allogeneic mixed lymphocyte reaction (MLR) and compared to that of normal (i.e., untreated) mice (Table 1). Mice burned and grafted at six hours (BE₆) showed 23.7 percent and 11.6 percent of the proliferative capability of normal T-cells on days five and seven post-burn, respectively. T-cells from animals grafted 24 hours post-burn (BE₂₄) were only 9.0 percent, 18.3 percent, and 7.4 percent as active as normals at days three, five, and seven, respectively. While the burn injury alone led to hyporesponsive T-cell cultures (Table 1, Burn), in both instances the added physiologic insult of escharectomy and grafting significantly depressed the cell-mediated immunity beyond that of the burn injury alone (p < 0.01).

To control for nonspecific suppression due to cell crowding the normal experimental cultures were set up at various total cell numbers (Fig. 1) and examined for reduced proliferation suggesting cell crowding. In none of the experiments discussed was the nonspecific suppression due to cell crowding.

![FIG. 2. Mortality rates observed for treated mice over a seven-day course. The treatments consisted of burn alone, burn with escharectomy and grafting at 24 hours post-burn (BE₂₄), burn with escharectomy and grafting at six hours post-burn (BE₆), excision controls (EC), or incision controls (IC).](image-url)
The hyporesponsiveness of the cell-mediated immunity observed on days three, five, and seven post-burn could have been due either to a passive depressed responsiveness of the helper T-cell population to an allogeneic stimulus or to a nonspecific active suppression by an activated suppressor T-cell circuit. To test for the presence of suppressor T-cells, T-cells from treated mice were added to normal MLRs (Fig. 1). Suppression of >50 percent was considered physiologically significant [10]. As seen in Table 2, activated T-suppressor cells could be demonstrated in the burn and six and 24 hours post-burn grafted animals on both days five and seven post-burn but not on day three.

**Immunosuppression Following Grafting Without Burn**

It was observed that the immunosuppression following thermal injury was compounded by the surgical procedure of escharectomy and grafting (Fig. 3). The ability of this procedure alone to induce immunosuppression was therefore studied by grafting mice without the antecedent burn. As with the burned and grafted animals the avascular graft of the excision control (EC) animals was dehydrated with spotty focal necrosis by day five post-graft, and a granulation response was visible across the dorsum of the mouse beneath the graft. Table 3 (EC) shows that at days five and seven post-burn the percentage of normal MLR response was 30.7 percent and 24.8 percent, respectively, which was comparable to that following the scald insult alone (Table 1, Burn). Activated suppressor T-cells could also be demonstrated on both days five and seven post-treatment (Table 4, EC), but, again, not on day three.

**TABLE 1**

|                | Day 3   | Day 5  | Day 7   |
|----------------|---------|--------|---------|
| Burn           |         |        |         |
| n = 6*         | 9.2 ± 3.8 | 33.2 ± 8.3 | 26.1 ± 2.1 |
| BE₆           |         |        |         |
| n = 6         | NA      | 23.7 ± 1.7 | 11.6 ± 2.5 |
| BE₂₄         |         |        |         |
| n = 12        | 9.0 ± 5.0 | 18.3 ± 1.1 | 7.4 ± 1.3 |

*See text for determination of n.
NA Not available


**Incision Control**

Finally, to control for the insults of anesthesia, incision, and suturing of the mice, a group of unburned mice were incised and sutured as described elsewhere. Unlike the excised and grafted animals described previously, the vascularity of the dorsal skin flap remained undisturbed in this incision control (IC) group. The incision wound healed completely and no changes were observed in the skin flap over seven days (i.e., no dehydration). Days three, five, and seven post-treatment MLRs from this group were not significantly different from normal cultures (Table 3, IC). The addition of T-cells from these mice to untreated control reactions was unable to suppress their proliferative capability (Table 4, IC). The enhanced response of the normal cultures observed after the addition of T-cells from treated animals was interpreted as an unchecked (i.e., unsuppressed) response of both the normal and treated T-cells to an allogeneic stimulus in the absence of suppressor T-cells. When compared to the normal MLR cultures containing an equal number of total cells (Fig. 1, S* × 3N), the activities were not significantly different (data not shown).

**DISCUSSION**

While recent reports have indicated that the very early excision of burn eschar may favorably alter the immunosuppression normally observed following thermal trauma [13], the results of this study showed that grafting of a full-thickness skin flap to a syngeneic recipient mouse was itself immunosuppressive and that this suppression was additive to that of the burn injury. Thus, the removal of large burn wounds followed by grafting cannot be expected to circumvent the post-burn suppressive episode.

Animals and humans subjected to burn injuries, trauma, or surgery respond by releasing several stress hormones including cortisol—a potent endogenous immunosuppressant. Continued physiologic stress with a persistently high serum cortisol has been correlated with an increased mortality [21]. However, Munster et al. [22] have demonstrated that cortisol is not required for the initiation or maintenance of post-burn or post-traumatic immunosuppression. By subjecting rats to bilateral adrenalectomies one month prior to a 30 percent TBSA burn or dermatome injury, they were able to lower serum cortisol levels to 4 µg/dl seven days post-trauma as compared to 90 µg/dl in nonadrenalectomized controls. The immunosuppression observed in each group was identical both temporally and quantitatively. Therefore, while cortisol may play a role in immunosuppression in an intact subject, it is secondary to and superimposed upon other independent factors.

Several observations have been made to support theories on an alternate mechanism of induction of post-burn immunosuppression. Without identifying the degree to which each is responsible for this post-traumatic event, each of the follow-

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**TABLE 3**

|               | Day 3     | Day 5     | Day 7     |
|---------------|-----------|-----------|-----------|
| EC n = 6*     | 44.6 ± 5.0| 30.7 ± 6.0| 24.8 ± 6.1|
| IC n = 3      | 107.1 ± 6.9| 90.8 ± 5.1| 90.4 ± 20.4|

*See text for determination of n.
ing imply that the removal of the burn eschar as soon as possible post-burn could avert the otherwise inevitable reduction in immunocompetence. Kremer et al. [14] have isolated and characterized a "burn toxin" from serum and skin of burned humans and animals which causes vacuolization of liver cells and irreversible mitochondrial damage with the uncoupling of oxidative phosphorylation in all cells including lymphocytes. The toxin is a trimerized lipid-protein complex formed by the heat-induced polymerization of naturally occurring precursors in the basal epidermal skin layer. Therefore, if the inciting factor for the suppression is a product of the wound, expedient removal of the source should prevent suppression.

Other investigators have observed a significant decrease in the ability of fixed macrophage of the reticuloendothelial system to clear foreign particles from the blood following thermal injury [9,10]. This presumably results in the presentation of large quantities of foreign and altered self antigens from the burned tissue to the immune surveillance system. It has been postulated that this "autoantigenemia" which cannot be sufficiently cleared by the impaired reticuloendothelial system contributes to the induction of an autoimmune response which is countered by a systemic nonspecific cell-mediated immunosuppression [4,20]. Again, very early removal of the antigen source should attenuate the antigenemia and thus the suppression.

More recently, Barbul et al. [15] have demonstrated in vitro and in vivo immunosuppressive effects of fluid and mononuclear cells extracted from surgical wounds. Each of these wound components was shown to suppress Con A- and PHA-induced lymphocyte blastogenesis, one-way allogeneic lymphocyte reactions, and allogeneic skin graft rejections at days five and seven post-trauma. The systemic nonspecific immunosuppression observed following major thermal trauma may be visualized as a disseminated presentation of the local reactions observed by Barbul. Thus, very early removal of this local influence would be expected to abrogate the systemic immunosuppression.

To assure a reproducible and significant immunosuppression following the burn insult in our murine model, a 30 percent TBSA scald wound was used [10]. Burns greater than 30 percent TBSA significantly increase burn shock-related mortality [17] while burns less than 30 percent TBSA do not assure consistent duration or degree of post-burn suppression [10,23]. Ideally, to investigate the effects of the removal of burn eschar on post-burn cell-mediated immunity, the burn injury should be followed by a simple escharectomy and closure of the wound by approximating the edges and suturing. Thus, the eschar, as a possible source of the initiating factor for post-burn immunosuppression, could be removed with minimal additional trauma. Unfortunately, primary closure of a wound of the magnitude employed was not possible without fatally compromising the animal's respirations and mobility. To attempt to alleviate this problem we burned two groups of mice over 30 percent TBSA as previously described and excised the burn eschar at four or six hours post-burn, leaving the escharectomy wounds open. This procedure resulted in 100 percent mortality within 12 hours by dehydration due to the extravasation of fluid through the exposed dorsums of the animals (data not shown). Therefore, all further experiments utilized a flap of full-thickness skin from a H-2 compatible (i.e., syngeneic) donor mouse to cover the escharectomy wound. Though the surgical trauma of grafting and the introduction of an avascular skin flap introduce significant variables to the escharectomy model, the inability of this procedure to prevent normal post-burn immunosuppression was an unexpected result. Thus, the focus of our investigation was changed from a study of the effects of escharectomy to a study of the effects of very early escharectomy and syngeneic full-
IMMUNITY FOLLOWING POST-BURN GRAFTING

thickness skin grafting on post-burn immunosuppression. However, as previously discussed, this model more closely approximated the clinical reality of human burn patient therapy.

The experiments of Hansbrough et al. have shown that the positive effects of simple escharectomy with a primary closure of the wound were dependent on the timing of the removal of the burned skin after the injury [13]. These experiments, however, utilized a burn injury of less than 20 percent TBSA to allow for a primary closure of the escharectomy wound. As previously discussed, the characteristics of the immunosuppression (in terms of degree and duration) following less severe burns are different from those of a burn of 30 percent or greater TBSA [10,23]. The suppressed cell-mediated immunity observed by Hansbrough et al. post-burn (reported as a decreased ear swelling following DNFB sensitization and challenge at 14 days post-burn) was nearly reversed following escharectomy at two hours post-burn, whereas mice subjected to escharectomies at 24 or 48 hours post-burn still experienced significant immunosuppression. When escharectomy and grafting were attempted in our study at less than four hours post-burn, the additional physiologic shock of a 30-minute grafting procedure resulted in a greater than 80 percent mortality rate within four hours post-surgery (i.e., shock-related deaths). Procedures performed six and twenty-four hours post-burn were relatively well tolerated and exhibited a quantitative pattern similar to that described above on the pathology of the cell-mediated immunity (Fig. 3). However, while the additional 18 hours of exposure to the burn eschar further suppressed the cell-mediated immunity, this difference failed to reach statistical significance ($p > 0.05$). Most important, neither procedure was able to improve the immunosuppression which follows major thermal trauma.

To investigate further the compounding effect of full-thickness skin grafting on the immunosuppression following the burn injury, several mice were subjected to the grafting procedure alone (i.e., without the antecedent burn). While no reports exist in the literature to allow a prediction of the effect of syngeneic skin grafting per se on cell-mediated immunity, it has long been recognized that post-traumatic immunosuppression is not unique to burn trauma. Similarly depressed nonspecific and specific immunity has been demonstrated following surgical manipulations [24,25,26]. Our experiments showed that the grafting procedure itself results in a pattern of depressed cell-mediated immunity which is both temporally and quantitatively equivalent to that of the burn insult alone on days five and seven post-treatment (Fig. 3). On day three post-treatment, the T-cell-mediated immunity of the excision control animals was observed to be substantially more responsive than that of the mice burned only or those burned and excised at 24 hours (Fig. 3). The suppressed immunity of these three groups on days five and seven post-treatment was shown to have been due to activated T-suppressor cells (Tables 2 and 4). We postulate, however, that the hyporesponsiveness of day three T-cells was due to impaired antigen presentation by the reticuloendothelial system as a result of an overwhelming antigenemia. Unlike the immediate injury of the burn, the skin graft deteriorated slowly over about five days. Thus, the difference between the burned animals and the excision control group on day three post-treatment could be seen as one of the degree of antigenemia on these days.

Since the incision control group of mice did not suppress on any day examined post-surgically (Table 3, IC) the suppression observed in the grafted mice must be attributed either to the skin removal process or some function of the dehydrated and necrotic graft. Though it was heat-induced, Kremer et al. [14] described their "burn toxin" as a lipid-protein complex which was formed after complete water loss (from
Dehydration of both the burn eschar and the skin graft by day five post-treatment naturally led to cell death and disruption. The hypervascularity observed deep to the eschar on day five offered a potential route of entrance into the systemic circulation of antigens released from the traumatized skin. Fixed macrophages of the reticuloendothelial system are normally charged with policing the body's circulation and removing foreign antigens. However, the depressed function of the reticuloendothelial system following burn [9,10] or surgical [24] trauma is incapable of efficiently carrying out this task. It has been postulated that the resultant antigenemia contributes to the autoimmune reactivity observed following severe burn trauma [4,20]. Under these conditions, immunosuppression can be interpreted teleologically as a means of "down regulating" a potentially harmful autoimmune response [20]. Thus, without directly challenging the above postulated immunosuppression induction circuit, we can say it is consistent with the observations of post-graft and post-burn suppression.

While it appeared that the suppression-inducing factor or factors was intrinsic to
IMMUNITY FOLLOWING POST-BURN GRAFTING

the skin, experiments by Hansbrough et al. have shown that though the subcutaneous injection of homogenized burn eschar into mice could induce immunosuppression, injections of homogenates of unburned skin were unable to induce any suppressive effects [13]. It, therefore, becomes necessary to suspect the contribution of a self antigen which is somehow altered by heat treatment or dehydration in the pathogenesis of suppression. Experiments are currently under way to investigate these possibilities.

As observed following burn trauma (both here and previously [2,4,10]), T-cell-enriched suspensions of days five and seven post-graft spleen cells were able to suppress the proliferative capability of normal allogeneic one-way mixed lymphocyte reactions (Table 4, EC). This cell-mediated suppression indicated the presence of an activated T-suppressor-cell circuit. Experiments by Kupper and Green and Kupper [4,10] have shown that the cell-mediated suppression observed on day seven post-burn is mediated by an Ly1+2* T-cell whose activity and phenotype are consistent with an activated T-suppressor cell [27]. The cell responsible for suppression on day five post-burn, however, was shown to have a Ly1+2*I-J* phenotype and required the presence of Ly2* cells to induce suppression. More recent unpublished data from the same laboratory have shown that two distinct Ly2* cells are required. Along with the Ly1+2* suppressor T-cell there is the requirement for a cyclophosphamide sensitive Ly1+2* transducer cell which relays the activating signal from the inducer to the Ly1+2* suppressor cell, thus completing the post-burn cell-mediated suppression circuit. Though the present work showed that escharectomy and skin grafting or grafting alone followed the same temporal and quantitative pattern of hyporesponsive immunity by active cell-mediated suppression as that described following severe thermal trauma (Fig. 3), careful characterization and phenotyping of this post-graft phenomenon is necessary before further parallels can be drawn. These experiments are also currently under way.

In summary, while recent reports support the theory that the very early removal of burn eschar may circumvent the problem of post-burn immunosuppression, the more clinically relevant post-burn therapy of early escharectomy with full-thickness skin grafting compounded the suppression caused by the burn. Furthermore, we showed that the surgical procedure of grafting and/or the graft itself leads to a "post traumatic" cell-mediated immunosuppression and that this suppression was additive to that caused by the burn injury. While it was not possible to monitor the appropriate hemodynamic parameters in our murine model, it would seem reasonable to assume that burned mice, with a total circulating fluid volume of approximately 2.5 ml, suffer a period of post-burn shock as seen in human burn patients. Initiating escharectomy and grafting procedures during the shock period (less than four hours post-burn) greatly increased the probability of a fatal outcome due to shock. As currently utilized in human burn patients, post-burn debridement and grafting fulfill infectious, homeostatic, and esthetic considerations, and we do not advocate that these procedures be delayed beyond the currently accepted time frames. While a longer exposure to the burn eschar may adversely affect the pathology of post-burn immunity in burn patients, it is apparent from this study that (1) regardless of the timing of the procedure, escharectomy and grafting could not prevent post-burn immunosuppression, and (2) attempts to perform these procedures during the shock period introduce unjustified risks. Thus, delaying excision and grafting until the patient is hemodynamically stable makes good clinical sense and does not risk losing the chance to abrogate suppression by an alternate means at an earlier time.
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