

# Cell-Mediated Immunity after Thermal Injury

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THE IMMUNOLOGICAL changes following burns have received considerable attention in the recent literature. It has been generally accepted that (1) Serum immunoglobulins become depleted in the postburn period,<sup>3,13</sup> (2) Skin allograft rejection is delayed in proportion to burn size,<sup>5,14</sup> and (3) Burn patients and animals become anergic to certain tests of delayed cutaneous hypersensitivity.<sup>4,15</sup> Other evidence on the immunological status of the burned host is more controversial. For example, the humoral response to systemically introduced antigens seems adequate<sup>1</sup> and the response of burn lymphocytes to phytohemagglutinin stimulation is in fact increased.<sup>6,10</sup>

In the combined clinical and laboratory study to be presented, we have attempted to look at some further aspects of cell-mediated immunity in burns. Specifically, we investigated the fate of internal as opposed to cutaneous allografts following burns; the primary and anamnestic responses to cutaneous allergens; the ability of burn lymphocytes to recognize foreign histocompatibility antigens; and the possible presence of any serum-transferable factor which might mediate a depression of cell-mediated immunity *in vitro*.

## Materials and Methods

*Rejection of Internal Allografts in the Burned Rat.* Adult Lewis and (Lew × BN) F<sub>1</sub> hybrid rats were used throughout. A 30% total body burn was inflicted on the recipient rat according to a previously described method, using a standard 10-second immersion scald burn;<sup>17</sup> then, following varying intervals of time, operation was performed according to a modification of the method of

Wheeler.<sup>18</sup> Recipient animals were anesthetized and the left kidney exposed through a subcostal incision. A fine slice of tissue was removed from the cortex of the kidney and, following cessation of bleeding, a slice of the spleen of the donor animal was implanted on to the host kidney surface and allowed to adhere without sutures. The kidney bearing the graft was then carefully replaced in the abdominal cavity and the abdomen closed in layers. All animals were sacrificed at 10 days and the spleen slice grafts scored grossly for color, size and vascularity. The kidneys were then excised, examined histologically (with the examiner having no knowledge of the specimen source), and scored by light microscopy for residual splenic architecture, lymphoid follicles, vascularization, necrosis and fibrosis. Using these criteria, a scoring system was devised from 0 (complete necrosis or fibrosis) to 6 (perfect survival), and this method was used to describe the state of the graft at 10 days. Figure 1 illustrates the histologic appearances of these spleen slice grafts at 10 days. The experimental design is detailed in Table 1. It will be noted that the principal experimental group (Group II) is so designed that a pure host-versus-graft reaction must take place. This was done in order to eliminate histological confusion by the occurrence of a bidirectional reaction.

A separate group of Lewis rats (n = 10 per group) received skin allografts from (Lew × BN) F<sub>1</sub> donors to establish skin rejection times for this particular strain. Recipients were inflicted a 15% scald burn placed as far posteriorly as possible on the back, then a 2 × 2 cm. partial thickness skin graft was placed on a surgically

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FIG. 1A. Compatible spleen slice graft (right) on renal cortex (left). Note cellularity of spleen and normal follicular architecture.

prepared bed anterior to the burn, and sutured in place. Control rats received skin grafts only. All grafts were read daily for signs of rejection, and the day of necrosis of 50% of the graft was taken as the day of rejection.

**Skin Tests in Burned Patients.** Eight patients, with burns ranging from 18 to 85% of the total body surface, were subjected to skin testing as follows. On admission, the inflammatory reaction was assessed by a skin-window technic, in which a small area of unburned skin was abraided with a scalpel, and a microscope coverslip secured over the area. Twenty-four hours later the coverslip was removed, fixed, and stained, and polymorphonuclear leucocytes and eosinophils counted. Results were classified as positive or negative. These patients were simultaneously tested with an intracutaneous injection

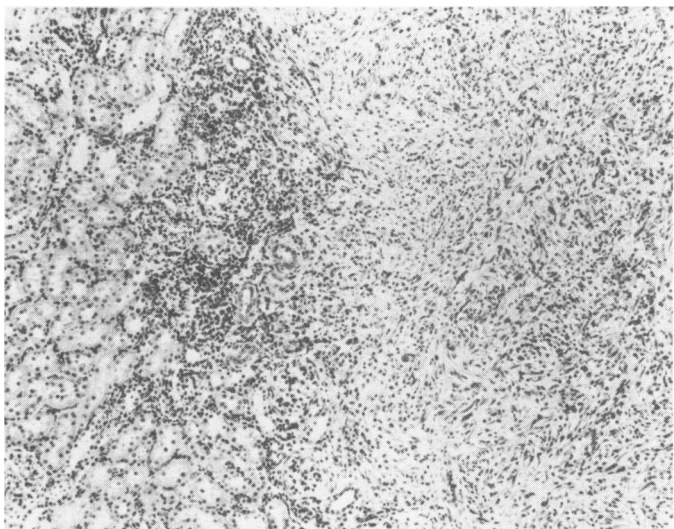


FIG. 1B. Incompatible spleen slice graft (right) on renal cortex (left). The spleen is avascular and infiltrated with fibrous tissue.

TABLE 1. *Experimental Design for Spleen Slice Allografts [F<sub>1</sub> refers to (Lewis × BN)F<sub>1</sub>]*

Group I— Compatible Grafts	Group II—Incompatible Grafts
Lewis → F <sub>1</sub>	F <sub>1</sub> → Lewis
F <sub>1</sub> → F <sub>1</sub>	F <sub>1</sub> → Lewis with burn 10 days pregraft
Lewis → Lewis	F <sub>1</sub> → Lewis with burn 4 days pregraft
F <sub>1</sub> → F <sub>1</sub> with 30% TBS burn 4 days pregraft	F <sub>1</sub> → Lewis with burn 1 day pregraft
Lewis → Lewis with 30% TBS burn 4 days pregraft	

of Monilia antigen, Trichomonas antigen, and Streptokinase-Streptodornase antigen. Since these tests are negative in persons with no prior exposure, a negative result provided no information. We retested all the patients at 2 weeks. The tests themselves do not induce a state of delayed hypersensitivity to the antigen, therefore a conversion from negative at first testing to positive at second testing can be interpreted as a transient loss of cell-mediated immunological memory to these antigens. Finally, all patients were sensitized on admission to Dinitrofluobenzene. This substance, in contrast to the previous group, does produce sensitization. Patients were challenged with DNFB again at 2 weeks. Failure to react to the challenge dose was interpreted as abolition of the primary immune response during the postburn period.

**Recognition of Heterologous Histocompatibility Antigen by Human Burn Lymphocytes.** We used a modification of the rat popliteal node assay.<sup>7</sup> The assay was originally designed to test the ability of parental rat lymphocytes, when injected into the hind footpad of the F<sub>1</sub> hybrid, to produce a Graft-versus-Host reaction in the draining popliteal lymph node. In this setting, we have already described a severe depression of the ability of spleen cells from injured rats to induce the reaction.<sup>12</sup> In the present experiments, 20 ml. of peripheral blood was obtained from the patients in the study at various intervals postburn. Leucocytes were separated by sedimentation, then 10<sup>6</sup> cells in 0.5 ml. medium were injected into the hind footpad of Lewis rats. One week later, the nodes were excised and processed for thymidine incorporation. Full details of the method are given elsewhere.<sup>12</sup> Results were expressed as the tritiated thymidine incorporation per minute by the rat popliteal node.

**Ability of Burn Serum to Depress *in vitro* Lymphocyte Transformation.** Peripheral lymphocytes were obtained from normal human volunteers, separated by dextran sedimentation, and adjusted to a final concentration of 10<sup>6</sup>/ml. Triplicate tubes were cultured for 6 days in a CO<sub>2</sub> ambient, in the presence of: (i) no additives, (ii) Monilia antigen 1:1000, 0.1 cc/culture tube, (iii) Monilia antigen with normal serum 0.1 cc, (iv) Monilia antigen with burn serum from our patients, 0.1 cc of undi-

luted serum and also 1:1, 1:2 and 1:4 dilutions. After 6 days, cultures were tagged with  $C_{14}$  thymidine and harvested. Since the reaction of peripheral lymphocytes to Monilia antigen depends on prior exposure, rather than expressing our results in absolute figures (disintegrations per minute), we expressed them as the mitotic index, 100% being the uptake of thymidine by the cultures containing Monilia antigen only. In this way, variations of reactivity due to differing states of sensitization of the lymphocyte donors was eliminated, and each series of cultures compared only to the activity of lymphocytes from the same donor sample.

Aliquots of serum from burned patients used in these experiments were set aside for serum cortisol determination. The serum cortisol concentration was compared with the percent inhibition produced in the Monilia-stimulated cell cultures by the same samples of serum.

### Results

*Rejection of Internal Allografts in the Burned Rat.* The results are listed in Table 2. The mean group scores for immunologically compatible groups, with or without thermal injury, range from 4.2 to 5.4, that is, good survival. Although none of these grafts reached the perfect score of 6, it is known that spleen slice grafts do not reach optimal follicular reorganization for 3 to 4 weeks using this technic,<sup>18</sup> and all our grafts were inspected at 10 days. The mean score of the incompatible groups ranged from 1.6 to 2.2 showing the severe rejection that would be expected from these Ag/B incompatible animals. Statistical comparison by analysis of variance of the scores of compatible groups is significant at the 0.1% level. The incompatible groups of rats with no thermal injury showed no significant difference in rejection from the incompatible groups grafted at 1, 4 or 10 days postburn ( $p > 0.5$ ). Thermal injury in this model, therefore, failed to depress the rejection reaction. By contrast, the skin allograft survival in burned rats of the same species was prolonged from a control value of  $6.4 \pm 0.3$  days to  $8.6 \pm 0.7$  days ( $p < 0.01$ ). This prolongation is small, but the burn inflicted on the recipient rats was only 15% of the body surface; the finding is therefore in accord with previous reports of increased skin graft survival following burning.

*Skin Tests in Burned Patients.* Of the eight patients tested, one with 85% burns survived long enough to allow initial testing only. Results are shown in Figure 2. It can be seen that two patients with burns of 18% and 20% respectively showed no diminution in their cutaneous responses at all. Two patients with 24% and 30% burns had a negative initial response to some of their immunological memory tests which turned positive at the second testing. Patients with 31, 38 and 52% burns remained negative to all skin tests. Although it is possible that all three patients had no prior contact with any of the three

TABLE 2. *Rejection of Spleen Slice Grafts Following a 30% Burn*  
( $F_1 = (Lewis \times BN)F_1$ )

Donor	Recipient	n	No. of Days Burned Before Grafting	Mean Score*
$F_1$	Lewis	6	No burn	1.6
$F_1$	Lewis	5	10	2.2
$F_1$	Lewis	6	4	2.0
$F_1$	Lewis	8	1	1.6
Lewis	$F_1$	5	No burn	5.0
$F_1$	$F_1$	5	No burn	5.4
$F_1$	$F_1$	6	4	4.7
Lewis	Lewis	6	No burn	4.2
Lewis	Lewis	6	4	4.2

\* Perfect survival = 6; complete rejection = 0

Analysis of variance: Within group differences for Group I,  $p > 0.5$

Within group differences for Group II,  $p > 0.5$

Group I vs. Group II,  $p < 0.001$ \*\*\*

antigens used, in a military population this is extremely unlikely. The same three patients also failed to sensitize to dinitrofluobenzene, indicating an abolition of the primary cell-mediated immune response. The patient with 85% burns was negative to all tests and showed no inflammatory response by the skin window method.

In these patients therefore, normal inflammatory and immunological responses were noted at a burn size of 20% or less. As the burn size increased, immunological memory to a cell-mediated antigenic stimulus, the ability to respond to a primary cell-mediated antigen, and finally, the inflammatory reaction, were progressively abolished.

*Recognition of Heterologous Histocompatibility Antigen by Human Burn Lymphocytes.* Results are shown in Figure 3. The rat popliteal node reaction was normal in patients with 18, 20, 24, and 30% burns. In the larger burns, it was severely depressed for up to 1 week post-burn, recovering to normal levels by two weeks. Since the reaction here was two-way, there having been no mitamycin-treatment or radiation of either human or rat

BURN SIZE %	ON ADMISSION				AT 2 WEEKS			
	Rebuck	SDSK	TRICHO	MONI	DNFB	SDSK	TRICHO	MONI
18	+	+	+	+	+	+	+	+
20	+	+	+	-	+	+	+	-
24	+	-	-	-	+	+	+	-
30	+	-	-	-	+	+	-	+
31	+	-	-	-	-	-	-	-
38	+	-	-	-	-	-	-	-
52	+	-	-	-	-	-	-	-
85	-	-	-	-	-	-	-	-

FIG. 2. Skin tests following burns.

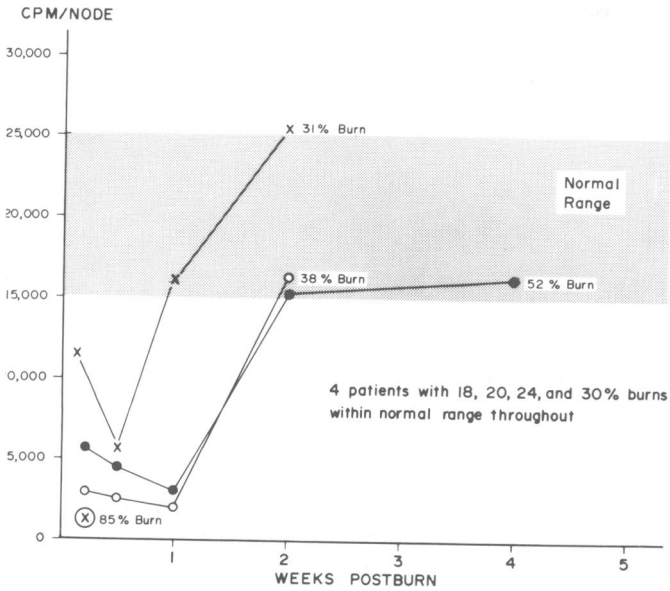


FIG. 3. Popliteal node assay.

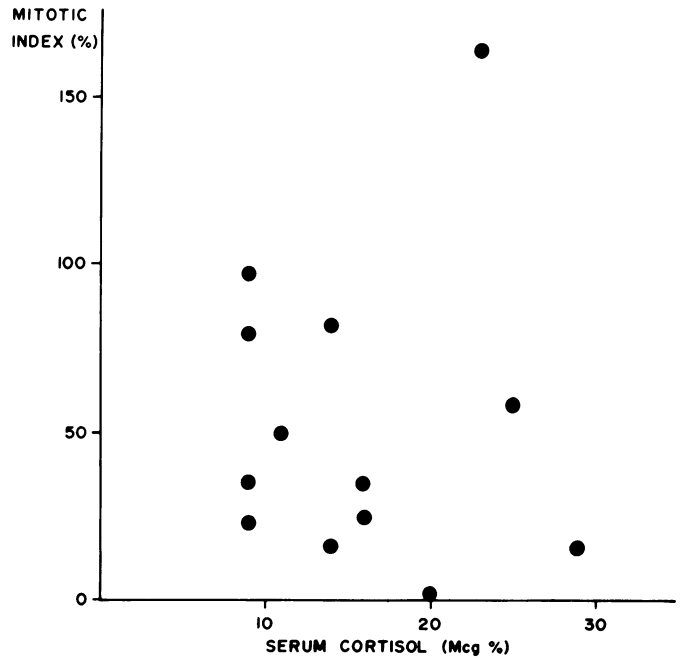


FIG. 5. Serum cortisol and inhibition of mitogenesis.

cells, these results strictly speaking cannot be interpreted as a failure of the human cells to react. However, there is evidence in the literature<sup>5</sup> that the histocompatibility markers of lymphocytes are not altered by thermal injury and presumably the rat cells should react to the human lymphocytes. In this case, one may conclude that the depression in the reaction as noted in our experiments was a reflection of the inability of the burned human cells to react against the histocompatibility antigens of the rat at least until one-way mixed lymphocyte reactions are investigated. This would agree well with other recent observations describing a suppression of the T-lymphocyte population postburn over the same time period.<sup>9,12</sup>

*Ability of Burn Serum to Depress in vitro Lymphocyte*

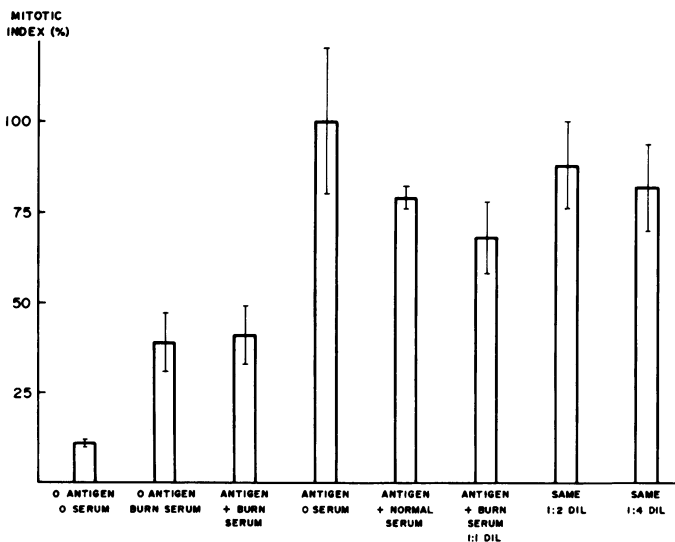


FIG. 4. Results of stimulation with Candida antigen.

*Transformation.* Results are shown in Figure 4. Taking the thymidine incorporation per minute of cultures of normal lymphocytes stimulated by Monilia, without serum, as 100% (actual count  $1751 \pm 393$ ), the following findings were noted. The addition of normal serum had no statistically significant effect on Monilia-stimulated cultures. The addition of undiluted burn serum reduced blast transformation to  $41\% \pm 7.9$  ( $p < 0.01$ ). The addition of a 1:1 dilution of burn serum produced a lesser depression at  $68\% \pm 10.4$  ( $p < 0.05$ ). Further dilution of the burn serum to 1:2 and 1:4 abolished the effect. Spontaneous transformation (no antigen and no serum added) was  $11\% \pm 1.2$ .

Serum cortisol values on the samples ranged from 9 to 23 mg./100 ml. A comparison of the per cent depression produced by the serum on Monilia-stimulated blast transformation with the serum cortisol concentration of the same sample is shown in Figure 5. Although the numbers are small, there appears to be no correlation between the degree of inhibition of mitogenesis and serum cortisol levels, and no correlation can be found by statistical analysis. Similarly, we were unable to document a correlation between burn size and the degree of inhibition of mitogenesis.

**Discussion**

Our findings of depression of cell-mediated immunity as indicated by abolition of certain delayed cutaneous hypersensitivity reactions are in agreement with previous reports.<sup>4,15</sup> It is interesting that the activation of the clone of immunologically responsive cells which are responsible for immunological memory appears to be depressed by a smaller burn than is necessary to depress the primary reaction. This is in contrast with the re-

sponse of humoral immunity, in which the secondary response is normal or even exaggerated postburn.<sup>2</sup> The relationship between burn size and the degree of depression of cell-mediated immunity, noted in the past,<sup>5</sup> is confirmed by our findings in the popliteal node assay.

The apparent normality of internal allograft rejection in the face of the above information is intriguing, but can probably be explained on a time-related basis. Since the time of sacrifice of our rats for scoring the spleen slice grafts was always 10 days, and the earliest grafts were placed one day postburn, these grafts had a minimum of 11 days in which to undergo rejection. In the rat, the ability of burn spleen cells to induce a Graft-versus-Host reaction is profoundly impaired immediately postburn, but recovers rapidly by 4 to 7 days.<sup>12</sup> This rapid recovery of T-cell function could allow enough time for histological rejection to occur by the 11th day. Skin allograft rejection in major burns, by contrast, is prolonged for a considerable time, sometimes weeks.<sup>2,5</sup> Two alternative explanations for this finding may be offered. First, antigenic processing from the skin may be less efficient than from a well-vascularized internal organ, and second, an organ-specific factor may be liberated from burned skin which depresses skin graft rejection. In the unburned dog, kidney grafts are more rapidly rejected than skin grafts.<sup>11</sup> Furthermore, a free F(ab')<sub>2</sub> fragment of immunoglobulin G has been isolated from the serum of burned patients.<sup>8</sup> Since this complement-independent serum fraction has an enhancing effect on organ allograft survival,<sup>16</sup> it is possible that the prolongation of skin allografts in burns is in the nature of an immunological enhancement.

We have demonstrated severe depression of *in vitro* lymphocyte transformation by a serum-transferable factor which, at least in our small series, does not appear to be cortisol. If this factor were of biological importance, one might expect a correlation between burn size and the degree of depression, a correlation which we were not able to demonstrate. We did not differentiate between second and third degree burns in this series, and it is possible that the depth of tissue destruction is of relevance in determining the release of the serum factor. Until further investigations are carried out, the implications of such a serum factor must of necessity be conjectural.

### Summary

A study of cell-mediated immunity on eight burn patients and groups of inbred rats has shown that immunological responses remain normal in burns smaller than 20% body surface. As the burn size increases, immunological memory and the primary cellular response become progressively abolished. In contrast to skin allograft rejection which is delayed by thermal injury, internal organ rejection proceeds normally. It is hypothesized that an organ-specific factor, possibly an enhancing

antibody, may be released from burned tissue. There is a serum-transferable factor present in burn patients which is capable of depressing the *in vitro* blast transformation of stimulated, normal human lymphocytes. Although this factor does not appear to be cortisol, its biological importance at present remains unclear.

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