T-Lymphocyte Function Following Burns: Dependence of Response on Antigenic Disparity and Size of Injury[1] (37262)

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The immunological effects of thermal injury are of importance to clinicians because burned patients are susceptible to infection by low-virulence organisms (1), and such infections are currently the commonest cause of a fatal outcome following such injury. Various measurements of cell-mediated immunity have been shown to be depressed in burned patients and animals. Skin allograft survival is prolonged (2), tests of delayed cutaneous hypersensitivity convert from positive to negative following injury (3), the peripheral T-cell population is depleted (4), and the *in vitro* mixed lymphocyte reaction of burn lymphocytes is partially abrogated (5). Increased burn size increases the degree of immunosuppression observed (2, 6). However, some contradictory findings have also been reported in the literature. The production of migration-inhibition factor by lymphocytes from burned animals appears to be normal (7). More important, several groups have adduced well-documented evidence that the phytohemagglutinin responsiveness of lymphocytes from burned patients and animals is markedly increased (8–10). To explain this apparent paradox, we conducted a series of experiments using the recently described popliteal node assay in the inbred rat (11), a sensitive index of thymic-mediated immunity.

Materials and Methods. We used combinations of Lewis with (Lew × BN)F_1 rats, which differ at the strong Ag/B histocompatibility locus, and F344 with (F344 × Lew)F_1 rats, which are compatible at the Ag/B locus. Rats weighed 180–200 g, were housed in individual cages, and allowed food and water *ad libitum*. Thermal injury was carried out according to a standard scald burn model (12) and burn size was calculated according to weight and surface area (13). To perform the assay, spleens from parental donors (either previously burned or controls which were only anesthetized) were excised at various times postburn, and 4×10^7 viable cells in 0.5 ml of TC 199 medium were injected into the right hind footpad of F_1 hybrid recipients. The left footpad, injected with medium only, served as an internal control. One week later the popliteal nodes draining the footpads were excised and weighed. The weight thus obtained is an index of the strength of the graft-versus-host reaction mounted by the injected cells against their host, and thereby a measure of T-cell competence in the donor.

Various other control groups were used to test the assay system. We subjected groups of both donors and recipients to 450 R total body radiation, observed their peripheral blood counts, and performed the assay when clear-cut evidence of hematological depression could be noted. Syngeneic injections of parent to parent and hybrid to hybrid injections were also performed in both Ag/B compatible and Ag/B incompatible animals.

Results. The results of the assay in syngeneic groups and in radiated control groups as well as the basic experimental parent to hybrid group are shown in Table I. Injection of parental cells into the F_1 hybrid caused an approximate 20-fold enlargement of nodes in the Ag/B incompatible animals, and a 3-fold enlargement in the Ag/B compatible animals. The injection of syngeneic cells caused a slight, but statistically insignificant, increase in node size. Radiation of either do-

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TABLE I. Basic Model for the Popliteal Node Assay.[a]

	Group I Ag/B incompatible P = Lew; F = (Lew × BN)F_1		Group II Ag/B compatible P = F344; F = (Lew × F344)F_1	
	Right node	Left node	Right node	Left node
F → F	6.6 ± 0.7	5.5 ± 0.8	13.3 ± 3.0	9.3 ± 2.5
P → P	7.2 ± 0.7	4.3 ± 0.8	8.2 ± 2.1	6.7 ± 0.7
P → F	131.1 ± 6.7[b]	6.6 ± 0.9	22.2 ± 3.5[b]	6.6 ± 0.4
Pr → F	78.7 ± 20.6	9.9 ± 0.5	13.4 ± 3.8	2.2 ± 1.9
P → Fr	59.4 ± 3.7	6.0 ± 1.4	5.0 ± 1.3	1.0 ± 0.3

[a] Pr, Fr = groups irradiated; results expressed as mg node weight ± SE; $n = 5$ per group.
[b] The two basic graft-versus-host groups, different from all other controls, $p < .01$.

nor or recipient effectively diminished the graft-versus-host reaction. These results conform to theoretical expectations and are in agreement with the findings of Ford (11).

The results of the assay when spleen cells were obtained from burned donors are shown in Table II (Ag/B incompatible) and Table III (Ag/B compatible). The experimental right nodes of these animals were compared to the unburned controls by Scheffe's method of analysis of variance. It can be seen that in the strongly incompatible combination, statistically significant increase in stimulation over unburned controls was seen at 7 days in the 18% burns, and 7 and 10 days in the 23% burns. In the weakly incompatible combination, in which all donors were burned 30%, highly significant depression was seen at 1 and at 4 days. Some depression persisted at 10 days but was no longer statistically significant. All other groups were within the significance limits of controls.

The left nodes, serving as internal control, showed only slight variation in size from group to group, generally in the direction of change taken by the experimental right nodes. This has been reported previously and explained by Ford in terms of lodgement of some donor lymphocytes in the contralateral popliteal nodes via the circulation (11).

Thus, in the presence of strong antigenic disparity and a small burn (18–23% size), increase in reactivity of donor T-cells was noted, returning to normal by 14 days postburn. In the strong disparity and large burn (30% size) group, the injury had no effect on the reaction. In the weak disparity–large burn group, the reaction was severely depressed but returned to normal by 10 days postburn.

Discussion. It is apparent that thermal injury can have either a stimulant or a depressant effect on cell-mediated immunity, according to the precise experimental setting. The mechanism of these changes can only be conjectured, although there is evidence that it is not steroid mediated (6, 14) but transferable by serum (6). We would like to postulate that the burned skin releases a factor, possibly a membrane-associated anti-

TABLE II. Assay with Cells from Burned Donors.[a]

Size (%) burn in donor	Day postburn splenectomized	Right node	Left node
18	1	125.6 ± 10.9	8.0 ± 1.0
	4	111.3 ± 22.0	7.4 ± 0.8
	7	224.3 ± 12.6[b]	10.8 ± 0.5
	10	209.8 ± 31.2	15.3 ± 0.7
	14	125.1 ± 7.6	6.5 ± 0.3
23	1	151.7 ± 13.5	10.3 ± 1.0
	4	114.8 ± 7.7	6.2 ± 0.8
	7	211.9 ± 12.2[b]	11.2 ± 0.7
	10	190.6 ± 9.2[b]	10.2 ± 0.7
	14	183.5 ± 30.2	9.2 ± 1.4
30	1	199.3 ± 23.1	10.4 ± 0.9
	4	167.2 ± 19.1	8.5 ± 0.7
	7	146.2 ± 14.8	5.6 ± 0.5
	10	158.1 ± 13.5	6.8 ± 0.8
	14	125.0 ± 14.0	8.4 ± 1.3

[a] P = Lew; F = (Lew × BN)F_1 (Ag/B incompatible); results expressed as mg node weight ± SE.
[b] Significantly higher than unburned P → F controls, $p < .05$.

TABLE III. Assay with Cells from Burned Donors.[a]

Size (%) burn in donor	Day post-burn splenectomized	Right node	Left node
30	1	12.6 ± 1.2[b]	6.3 ± 0.5
	4	14.6 ± 1.1[b]	6.6 ± 0.9
	10	17.3 ± 2.6	6.0 ± 0.4
	14	21.5 ± 3.2	7.1 ± 0.6

[a] P = F344; F = (Lew × F344)F_1 (Ag/B compatible); results expressed as mg node weight ± SE; $n = 5$ per group.

[b] Significantly lower than unburned P → F controls, $p < .01$.

gen, which is capable of triggering blast transformation by thymic-dependent lymphocytes. This may explain the marked increase in phytohemagglutinin responsiveness reported in the literature, since this mitogen is known to act preferentially on T-lymphocytes. In the presence of a small burn and the release of small amounts of antigen, the large pool of T-cells available to respond to a strongly different histocompatibility antigen may be stimulated; with the release of more antigen, the reaction would become partially blocked. With a large burn and a small responding T-cell population, in the Ag/B compatible animal, antigenic overload paralysis would occur. The 10–14 day postburn interval required for this antigenic activity to disappear corresponds well both with the timetable of increased phytohemagglutinin responsiveness, and with the normalization of skin tests of delayed hypersensitivity previously mentioned.

Summary. Using the Ford popliteal node assay, we found that the ability of spleen cells from burned rats to induce a graft-versus-host reaction in F_1 hybrid recipients varied according to burn size and the histocompatibility difference between donor and recipient. It is postulated that a factor, possibly related to histocompatibility antigens, may be released from the burn site, capable of stimulating T-cells in small doses and of inducing antigenic overload paralysis in large doses.

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