Lymphoid Subpopulation Changes after Thermal Injury and Thermal Injury with Infection in an Experimental Model

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Subpopulation analysis of peripheral blood lymphocytes is a frequently used measure of immunocompetence. Yet, little is known about the lymphocyte subpopulations in the circulation and lymphoid organs after severe trauma. Blood, spleen, and lymph node (LN) subpopulations were compared in a rat model of burn injury (B) and burn injury with infection (BI). B and BI rats received 30% total body surface scald burns. Infection was induced by seeding wounds with *Pseudomonas aeruginosa*. Subpopulations were identified by flow cytometry 48 hours after burn. Helper lymphocytes were selectively depleted from the circulation of BI but not B animals, which caused the ratio of helper to suppressor cells (HSR) in BI animals to decrease significantly compared with the unburned controls. Both LN helper and suppressor cells were decreased in BI animals and the HSR was unchanged, but a selective reduction in suppressor cells in B LN increased the HSR relative to unburned controls. Spleen subpopulations were unchanged for both B and BI groups. Subpopulation changes after trauma and infection were different for each tissue examined.

INFECTION STILL ACCOUNTS for a large fraction of the mortality observed in patients with large burns.[1,2] The susceptibility of burned patients to infection by organisms that are ordinarily nonpathogenic suggests an acquired defect in host defense mechanisms. Many aspects of host defense have been examined in an attempt to define this underlying defect, and many cellular and humoral aspects of host defense have been found to be abnormal.[3-8] However, none of the abnormalities described can be pinpointed as the cause of the observed increased susceptibility to infection.

Several conditions that present clinically with increased risk of infection are accompanied by relative changes in lymphocyte subpopulations,[9] usually described as a decrease in the ratio of T-helper to T-suppressor cells (HSR) in the circulation. The shift in this ratio may reflect a change in the balance between immune stimulation and suppression. An inappropriate change in this regulatory balance could lead to decreased resistance to infection.

Lymphocyte subpopulation changes are usually measured in lymphocyte samples from peripheral blood. Peripheral blood lymphocytes are a relatively small fraction of the total lymphocyte pool and may not present a valid picture of the immunological state of the individual. This mobile pool of cells might be expected to fluctuate more widely in response to stress or infection than the lymphocyte populations in the relatively immobile lymphoid tissues. Additionally, the accuracy of measurements of peripheral blood lymphoid cells can be impaired by atypical leukocyte populations, which are often found in the circulation of trauma victims several days after severe injury.[10] Sampling of a greater range of lymphocyte sources, such as spleen and lymph nodes, may give a more general picture of immunocompetence than assessment of peripheral blood alone.

We have analyzed the changes in lymphoid cell populations that occur after either burn injury or burn injury complicated with infection in a well-defined burn and burn-infection model.[11] The results show that relative increases in the proportion of suppressor lymphocytes relative to helper lymphocytes (decreases in the HSR) do

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In conducting the research described in this paper, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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not occur in the blood, spleen, or lymph nodes of infection-susceptible but uninfected burned animals. Decreased HSR was observed only in the blood of burned animals with infection.

Methods

Male albino Sprague-Dawley rats (300–400 g) were randomly assigned to one of three groups: an unburned control group, a burned group, or a burned group with infection. All rats were anesthetized with pentobarbital (IP, 1 mg/100 g body weight), and those in the burned groups were shaved, placed in a plastic mold that defined the burn surface area, and subjected to a 30% total body surface full-thickness burn by 10-second immersion in boiling water.[11] Infection was induced by placing 1 ml of a 16-hour broth culture containing approximately 10^8 *Pseudomonas aeruginosa* (strain 59-1244) on the burn wound within 1 hour of scalding.[12] At sacrifice, 48 hours after infection, the rats were anesthetized with pentobarbital and exsanguinated by opening the body cavity and bleeding from the hepatic vein. Subsequently, blood, spleen, and mesenteric lymph nodes were taken for cell analysis.

Peripheral blood leukocyte counts were made using a Coulter Counter (model ZM, Coulter Electronics, Hialeah, FL), and differential analyses were performed on blood smears. Spleen cells were obtained by disrupting the spleen and passing the cell suspension through a loosely packed cotton wool column to remove debris. The lymphoid cells from blood and spleen were separated from other cells by centrifugation over a Ficoll-Hypaque density gradient.

Lymph nodes were trimmed of excess tissue and minced with scissors over a 60-mesh stainless steel screen. The cells passing through the screen were passed through a loosely packed cotton wool column to screen out extraneous pieces of tissue. More than 95% of the lymph node cells obtained in this manner were lymphocytes and these cells were used without further purification. The isolated cells from each tissue were washed and samples of the cells were used to prepare slides for differential analysis. The remaining cells were stained with appropriate lymphocyte mouse monoclonal reagents obtained from Pel-Freez (Roger, AZ). The monoclonal reagents attached to the cell surface were further bound with affinity-purified, fluorescein-labeled goat anti-mouse IgG (Fab2' fragments) as a second step reagent.

The fluorescently stained cells were then analyzed, using a Fluorescence Activated Cell Sorter, model 400 (Becton Dickinson, Mountain View, CA), modified by addition of a 90-degree light scatter PMT and a consort 40 data analysis system. For each sample, 5000 cells were analyzed and the numbers of cells fluorescently stained with T lymphocyte (W3/13), suppressor/cytotoxic (OX-8), or helper/inducer (W3/25) reagent were determined. A negative control using a monoclonal antibody to human T cells (which had no specificity for rat cells) was run with each cell preparation to determine an appropriate cutoff to distinguish negative from positive cells. The positive cutoff was set at a point defining the upper 2% or less of the background control and the number of background control cells beyond the cutoff was subtracted from each sample. Contamination by nonlymphoid cells was monitored by analyzing forward and 90-degree scattered light. Those cells with light scatter intensity not typical of normal lymphocytes were not analyzed (gated).

The proportion of the positive cells for each of the subsets were compared among the three animal groups. Statistical analysis consisted of a pairwise analysis of means (t-test) with a Bonnferroni adjustment, performed on a VAX 11/780 (Digital Equipment Corporation, Maynard, MA) using program P7D (BMDP Statistical Software, Los Angeles, CA).

Results

Light scatter analyses of cells from each tissue were compared with morphologic analyses of cells on Wright's stained preparations to determine the effectiveness of the light scatter analysis as a measure of nonlymphoid cell contamination. Ficoll-Hypaque purified blood cells from burned animals showed no significant change in either light scatter characteristics or Wright's stain morphologic characteristics when compared with unburned controls (Table 1). Blood cells from burned animals with infection, however, showed a significant decrease in the proportion of cells ($p < 0.01$) with scatter characteristics typical of normal lymphocytes. This decrease correlated with a decrease in the proportion of cells with lymphocyte morphologic characteristics and an increase in the proportion of cells with morphologic characteristics of immature granulocytes or atypical mononuclear cells. There was no significant change from control in the light scatter characteristics or cellular morphologic characteristics of lymph node or spleen cells from burned or burned animals with infection.

Surface antigen analysis was performed on blood mononuclear cells that exhibited light scatter typical of normal lymphocytes (Fig. 1). The proportion of helper lymphocytes was decreased, whereas suppressor/cytotoxic lymphocytes were increased in the blood of burned rats with infection relative to control, but not in the blood of animals subjected to burn alone. This relative shift in subpopulations caused the HSR of circulating lymphocytes to decrease significantly ($p < 0.01$) in burned animals with infection compared with controls. Lymph node cells from burned animals with infection (Fig. 2) consisted of a significantly smaller proportion of T-lymphocytes (Pan-T) than uninfected animals as well

TABLE 1. *Differential Analysis of Ficoll-Hypaque Preparations by Light Scatter and Wright's Stain Morphology**

Cell Source/Group	Cells ± SD (% in "Lymphocyte" Gate)	Significance Level	% Lymphocytes (Wright's Stain)
Blood			
Control	77.6 ± 11.01	ns	91.0 ± 9.9
Burned	73.8 ± 8.8	ns	87.0 ± 11.4
Burned-infected	56.4 ± 16.3	(vs. control $p < 0.01$)	74.9 ± 16.4
Spleen			
Control	66.2 ± 12.0	ns	99.5 ± 1.0
Burned	69.2 ± 13.3	ns	98.7 ± 1.8
Burned-infected	61.3 ± 18.7	ns	95.8 ± 11.4
Lymph node			
Control	91.8 ± 4.9	ns	100 ± 0
Burned	89.9 ± 7.5	ns	99.9 ± 0.1
Burned-infected	92.0 ± 8.1	ns	99.8 ± 0.5

* Cells were analyzed for light scatter characteristics by flow cytometry and the number of cells falling within the lymphocyte "window" determined. A small aliquot containing approximately 10^5 cells was used to make a cytocentrifuge slide that was stained using Wright's stain and examined for the relative number of lymphoid and nonlymphoid cells under light microscopy.

as reduced proportions of both helper and suppressor lymphocytes. In burned uninfected animals the proportion of suppressor lymphocytes was lower than that of control animals, but the proportion of helper and Pan-T positive cells was not significantly different. Spleen cells from infected animals contained a small subpopulation of cells (5–10% of the total) that bound monoclonal antibody control reagents. Control reagents had the same mouse immunoglobulin isotype as the monoclonal reagents, but had no reactivity to rat cells. Therefore, the data depicted for spleen cells (Fig. 3) from infected animals may overestimate the true value for one or more of these populations by 5–10%. However, there was no significant difference in any of the three T-lymphocyte subpopulations or the HSR in the spleens of burned or burned animals with infection compared with unburned controls.

A summary of the HSR determined for each tissue is depicted in Figure 4. Infected animals show more variation in the ratio than the other groups, having a decreased ratio in blood but an increased (although statistically not significant) ratio in lymph node. The HSR increased in the lymph node even though both helper and suppressor/cytotoxic subsets decreased, because there was a disproportionate decrease in suppressor cells. The decrease in suppressor cells present in the lymph nodes of burned animals caused an increase in the HSR. This was the only difference in any of the lymphocyte subpopulations induced by the burn injury alone. The HSR determined in spleen cells was not significantly different from control in either of the experi-

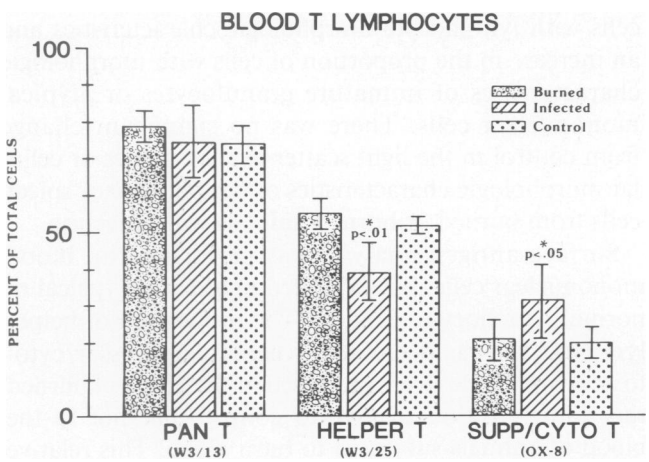

FIG. 1. The proportion of Ficoll-Hypaque purified blood cells positive for three T-lymphocyte surface antigens. Cells from burned and burned-infected and control rats were analyzed by flow cytometry for each of the three major T-lymphocyte surface antigens. The number of cells staining positively for each antigen is expressed as the mean percentage of total cells analyzed ± SD (N = 10).

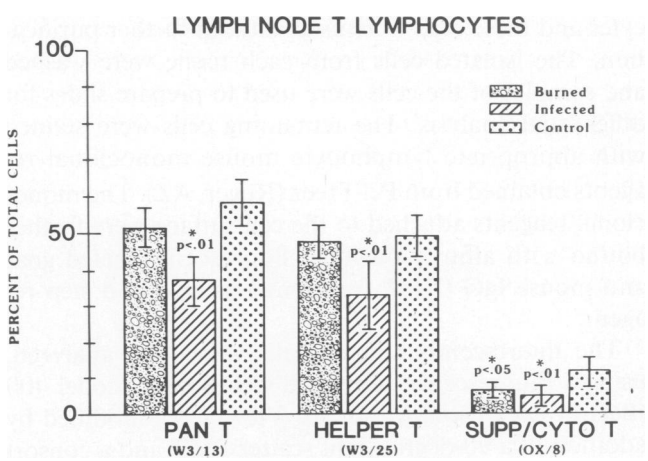

FIG. 2. The proportion of lymph node cells positive for the T-lymphocyte surface antigens. Conditions are the same as for Figure 1.

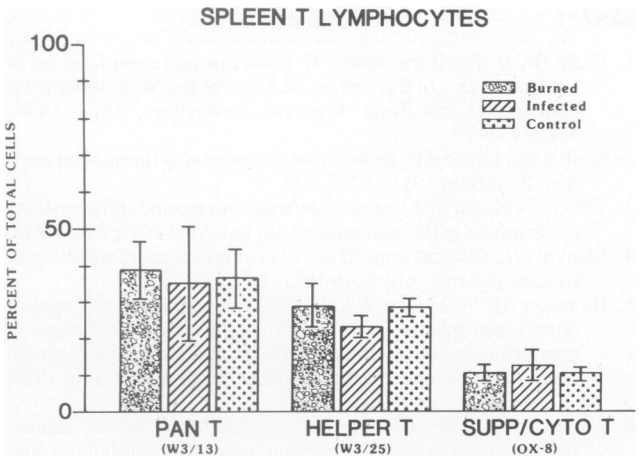

FIG. 3. The proportion of Ficoll-Hypaque purified spleen cells positive for the T-lymphocyte surface antigens. Conditions are the same as for Figure 1.

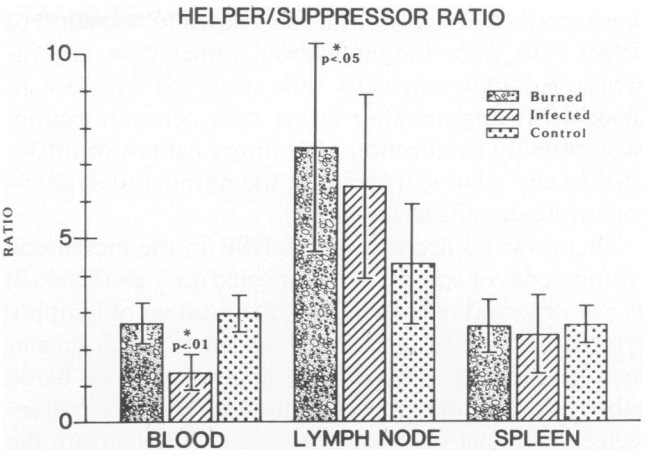

FIG. 4. The ratio of T-helper to T-suppressor/cytotoxic lymphocytes from blood, spleen, and lymph node for control, burned, and burned-infected rats. Conditions are the same as for Figure 1.

mental groups even though the number of lymphoid cells in the spleen was decreased sharply in the infected animals (data not shown).

The changes induced by burn injury alone and burn injury with infection are summarized in Table 2. A decrease in the suppressor subpopulation in the lymph nodes was the only significant subpopulation change for the infection-susceptible, but uninfected, burned animals. In the infected animals, proportional decreases were observed in all lymph node T cell subpopulations. In the blood of infected animals, the proportion of helper lymphocytes decreased, whereas the proportion of suppressor lymphocytes increased. The only changes in HSR were a decrease in the blood of infected animals and an increase in the lymph node of burned animals without infection.

Discussion

Other studies have been interpreted as showing that burn injury increases the relative proportion of suppressor cells (decreases HSR) in the burned host. It has been implied that such a shift in subpopulations decreases the capacity of the host to mount an immune response and thereby decreases resistance to infection. It is important to note that the changes in HSR are relative and that an absolute increase in the number of suppressor cells has not been reported. Using a mouse model, Zapata-Sirvent and Hansbrough[13] found a decreased spleen cell HSR to be correlated with decreased skin hypersensitivity to dinitrofluorobenzene 10–14 days after thermal injury. In a later study[14] Zapata-Sirvent et al. described a correlation between decreased hypersensitivity and mortality after infection induced by cecal ligation and puncture. In a rat burn infection model we have found that a decrease in the HSR in peripheral blood occurred only in the presence of infection and not as a consequence of injury *per se*.[15] In human burn patients a correlation between a decreased HSR and increased susceptibility to infections has been reported in patients with large burns by McIrvine et al. and Antonacci et al.[8,16] They reported decreased HSR in patients with burns covering more than 30% of the total body surface. In one study,[8] decreased helper/suppressor ratios occurring more than 14 days after burn were positively correlated with the occurrence of fatal sepsis. As in most clinical studies it is difficult to determine whether lymphocyte subpopulation changes are related to the cause of infection or are an effect of infection.

In the current study, the blood HSR measured 48 hours after injury and infection was decreased in infected animals. In uninfected burned animals, however, the HSR was not significantly different from control. Since infected animals have decreased circulating lymphocytes,[15] the change in proportion of helper and sup-

TABLE 2. *Comparison of Lymphocyte Subpopulations and HSR from Blood, Lymph Node, and Spleen**

Tissue	Group	Pan T	Helper	Suppressor	H/S Ratio
Blood	Burned	ns	ns	ns	ns
Blood	Infected	ns	−32.3‡	+19.2†	−53.7‡
Lymph node	Burned	ns	ns	−43.8†	+72.1†
Lymph node	Infected	−36.2‡	−32.9‡	−56.9‡	ns
Spleen	Burned	ns	ns	ns	ns
Spleen	Infected	ns	ns	ns	ns

* Values shown are percentage changes from the unburned control.
† Significant at 0.05 level.
‡ Significant at 0.01 level.
ns = change not statistically significant.

pressor cells due to infection was related to depletion of helper cells from the circulation, rather than any increase in suppressor cells. The observed decrease in blood HSR appears after rather than before infection. Susceptibility to infection after injury cannot be attributed to any relative increase in the number of suppressor/cytotoxic cells in the blood.

There was no decrease in the HSR in the mesenteric lymph nodes or spleens of the infected animals (Table 2) as was observed in blood. The total number of lymphocytes in the lymph nodes and spleen is much greater than in peripheral blood. The changes seen in blood subpopulations may represent transient depletion of selected subsets of lymphocytes as they migrate from the peripheral circulation. The subsets of the lymphocytes in the circulation are apparently subject to wider fluctuations in response to infection than those that occur in the total lymphoid population. The lack of uniformity among subpopulation shifts in the different tissues demonstrates that each of the lymphoid tissues acts, to some extent, as an individual compartment rather than as part of a common reservoir of lymphoid cells.

Although relative changes in lymphocyte subpopulations do not appear to accompany the increased susceptibility to infection after burn injury, subpopulation analysis of peripheral blood may have clinical use as an indicator of existent sepsis, since HSR appears sensitive to the presence of infection. That possibility awaits further study. Since the immune response results from a complex interaction among several subpopulations of cells, measurement of cell function rather than simple enumeration of subpopulations is more likely to yield information about the nature of the defects in host defense after burn injury.

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