Leukocyte Responses to Injury

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Injury elicits a response from all cells of the immune system in which cytokines and other metabolic products of activated leukocytes can act either beneficially to provide for enhanced host resistance or deleteriously to depress the function of remote organs and cause what has been termed systemic inflammation. These at times antithetical responses of leukocytes that appear to integrate postinjury changes in the neuroendocrine, immune, and coagulation systems have been implicated as principal causative factors in multiple systems organ failure. Numerous investigators have evaluated a variety of therapeutic agents to prevent and control infection by restoring leukocyte function, while others have evaluated antagonists and monoclonal antibodies as a means of controlling the exaggerated and persistent actions of leukocytes and cytokines caused by systemic inflammation. The redundancies of the cell populations and the cytokines and other metabolites produced by the cells predictably limit the effectiveness of any single agent and make clinical evaluation of such agents difficult.

Clinical and laboratory studies during the past two decades have documented that changes in leukocyte number and function can represent both the causes and the effects of infection, sepsis, and injury in surgical patients. Injury elicits a response from the immune system that is proportional to the magnitude of the insult and causes impaired function in some cells while sensitizing other cells so that a second insult will trigger an exaggerated and prolonged response. The cytokines and other metabolic products of activated leukocytes are also recognized as mixed blessings which, depending on magnitude and time of production, can act either beneficially to provide or enhance host resistance and thereby prevent or control infection, or deleteriously to depress the function of remote organs and cause "systemic inflammation" associated with cellular death and tissue injury.

Neutrophils are distinguished by their ready availability, short life span, rapid replacement, high phagocytic activity, rapid oxidative burst following stimulation, and potent array of oxidative metabolites and digestive enzymes. The sequence of neutrophil activation and subsequent release of oxidants and enzymes is extremely complex and subject to a wide variety of regulatory mechanisms. When appropriately stimulated by opsonized microorganisms, antigen antibody complexes, chemotactic factors or other stimuli, neutrophils activate a reduced form nicotinamide adenine dinucleotide phosphate oxidase-dependent respiratory burst resulting in the univalent reduction of molecular oxygen to superoxide anion. Superoxide is then dismuted either enzymatically or spontaneously to hydrogen peroxide. The newly generated hydrogen peroxide is utilized by metalloperoxidases, such as myeloperoxidi-
In the past two decades, numerous studies have described the functional characteristics of neutrophils as related to infection. Most early studies were conducted in patients or animals with thermal injury because of the relative ease of quantifying the injury, ie, percentage of total body surface burned. The finding of defective bactericidal function in the setting of normal phagocytosis in polymorphonuclear leukocytes (PMNs) from patients with burns was reported by Alexander and Wixson in 1970.

In 1974, Warden et al described suppressed chemotactic activity of neutrophils from patients with burns. Such findings led to formulation of the hypothesis that trauma-induced hypofunction of PMNs was partially responsible for the patient's increased susceptibility to infection. This hypothesis was further supported by the findings that the ability of PMNs to kill bacteria was even more deficient in patients who developed infection following trauma. Warden and colleagues, in a modification of the technique described by Boyden, reported a marked decrease in chemotaxis to activated serum in neutrophils from burned patients. Of note, the chemotactic activity was inversely related to burn size and the incidence of infection. Further in vivo evidence that such defects were related to infection was supplied by Balch, who reported a decrease in the immigration of neutrophils into skin windows implanted in unburned skin of thermally injured patients. Moreover, Yurt and Shires reported that systemic exposure to an exogenous chemotaxin (f-met-leu-phe [FMLP]) resulted in an increased susceptibility to infection in a rat burn model. They concluded that the chemotaxis of PMNs and caused nonspecific or indiscrete PMN margination in all capillary beds.

**EVIDENCE FOR SYSTEMIC ACTIVATION**

In 1986, Moore and coworkers reported that resting neutrophils from thermally injured patients were systemically activated, as indexed by increased expression of complement surface receptors. They concluded that the alterations of depressed chemotaxis, impaired bactericidal activity, decreased phagocytosis, and loss of lysosomal enzymes actually represented changes in neutrophil function due to in vivo exposure to stimuli that were abnormal in magnitude and resulted in systemic rather than localized activation. Using immunofluorescent techniques they serially studied expression of the complement opsonin receptors CR1 and CR3 as well as neutrophil chemotaxis in response to zymosan-activated serum, a source of C5A, in neutrophils from burned patients. Like other investigators, they found that for the first 3 postburn weeks, chemotaxis was depressed. Surprisingly, they found that neutrophil expression of CR1 and CR3 receptors were inversely correlated with the chemotactic index. Normally, exposure of neutrophils to a chemotactic attractant results in directed migration and an immediate and sustained increase in the number of cell surface receptors for complement proteins. The role of these receptors is to facilitate phagocytosis of bacteria and other particulate matter that is coated with C3b and iC3b. Increased expression of these complement receptors is an indication of cellular activation.

Other evidence for in vivo neutrophil activation has been reported by Dobke et al who found that resting neutrophil oxygen uptake was significantly increased in burned patients than in normal controls. We examined the resting cytosolic oxidative activity of neutrophils from 23 burned patients for 6 weeks following injury. Neutrophils were loaded with dichlorofluorescein, a compound which, when oxidized, exhibits strong fluorescence that serves as an index of cytosolic peroxidative activity. Cells were studied prior to and following stimulation with phorbol myristate acetate. The patients' neutrophils had significantly greater fluorescence prior to stimulation than those from control subjects (Figure 1). This indicated a baseline increase in cytosolic peroxidative activity that we interpreted as yet another manifestation of in vivo activation. Following stimulation with phorbol myristate acetate, the neutrophils had a normal or increased oxidative burst potential. Thus, it appears that following thermal injury as well as nonthermal trauma, neutrophils are systemically activated.

**OXIDANTS AND OXIDATIVE ACTIVITY**

The loss of neutrophil chemotactic function appears to be multifactorial and may be segregated into specific and nonspecific causes. The majority of studies have documented defects in chemotaxis as related to specific che-
motaxins. The downregulation of C5A receptors reported by Moore et al. was also noted by Solomkin and colleagues. They reported an average loss of 75% of ligand-binding activity from neutrophils isolated from patients with major thermal injury at a time when chemotaxis in response to C5A was maximally depressed. A decrease in C5A receptor expression would explain this specific defect in chemotaxis. However, it has also been noted that neutrophils in response to complement by-product exposure may exhibit an increase in FMLP receptors while at the same time demonstrating a decreased chemotactic response to an FMLP gradient. This nonspecific deactivation represents a nonspecific loss of function that cannot be attributed to receptor ligand binding.

The nonselective chemotactic dysfunction is thought to result from the activity of reactive oxygen species affecting cell components that are required for migration. Increased hydrogen peroxide production such as that which occurs in PMNs from patients with trauma and as indexed by an increase in cytosolic oxidative activity in neutrophils from burned patients has been proposed as a mechanism by which auto-oxidation of neutrophil locomotory machinery is possible. The oxidation of cell components required for migration was first proposed by Nelson and colleagues in 1979. They noted that neutrophils from patients with chronic granulomatous disease (a defect in which cells cannot produce oxygen metabolites) lose chemotactic function attributable to a loss of only the population of receptors specific for the stimulating chemotaxin. In another article, the same authors noted that normal neutrophils exposed to stimuli in the presence of colchicine, which blocks oxygen metabolite production, similarly lost chemotactic function only to specific receptor populations dependent on the chemotactic stimulus.

Maderazo and colleagues provided indirect support for the occurrence of auto-oxidation in neutrophils from patients with blunt trauma. They documented increased resting hydrogen peroxide production in conjunction with dysfunction of the microtubular apparatus as indexed by concanavalin A capping assays. These results suggested that microtubular assembly was inhibited or microtubular disassembly occurred in PMNs from patients with trauma. In vivo oxidizing agents prevent microtubular assembly and inhibit the normal uniform surface distribution of concanavalin A in human PMNs. Although Maderazo et al. were unable to document changes in the level of glutathione in the PMNs from patients with trauma, they noted a significant decrease in serum and cellular ascorbic acid and α-tocopherol levels. These investigators randomized patients with trauma to receive replacement antioxidant therapy and documented significant improvement in PMN chemotactic function. Finally, preliminary studies in this and other laboratories documented abnormalities in actin polymerization and depolymerization that may also contribute to the nonspecific decrease in granulocyte chemotaxis.

Activation of neutrophils also leads to an increased synthesis of PGE1, Bjornson and colleagues showed that PGE1 elevates intracellular cyclic adenosine monophosphate levels. In a guinea pig model of burn injury in which PMNs exhibited a marked bactericidal defect, these investigators were able to improve bactericidal activity by inhibition of cyclo-oxygenase activity with nonsteroidal anti-inflammatory drugs. The enhancement of bactericidal activity mediated by nonsteroidal anti-inflammatory drugs was fully counteracted by addition of purified PGE1, theophylline, and cyclic adenosine monophosphate. They concluded that the bactericidal defect in PMNs induced by thermal injury is related to the elevation of the cyclic adenosine monophosphate level and that PGE1 plays a significant role.

CANDIDA FOR ACTIVATORS

It is well known that the exposure of normal human PMNs to a variety of chemotactic factors that include activated serum, C5A, and FMLP, as well as opsonized zymosan results in enhanced cellular chemiluminescence and superoxide production, increased microtubular and microfilament assembly, and increased adherence as well as increased expression of complement receptors. These effects have been noted to last at least 2 hours, indicating a permanent change in cellular metabolism, surface membranes, or the cytoskeleton. The findings by Lowbury and Ricketts in 1957 of complement abnormalities following trauma, which were corroborated by the report of Gelbard et al. in 1982 documenting activation of complement by the alternative pathway following burns in mice and humans, raised the possibility that complement by-products may be responsible for systemic neutrophil activation. The increased neutrophil aggregation that correlated with complement activation is now known to be secondary to alterations in the expression of cell surface adherence receptors.

Davis and colleagues extended their previous study in a second group of thermally injured patients in whom they found increased neutrophil CR1 and CR3 expression that persisted for up to 50 days following injury and correlated with plasma levels of complement by-products. Despite the overall correlation between complement products and complement receptor expression, peak complement receptor expression occurred between postburn days 0 and 5 while peak generation of complement activation products occurred after postburn day 6. These data were interpreted as indicating that a second neutrophil-activating substance might be present early in the postinjury course.

Lipopolysaccharide (LPS) has been suggested as a potential candidate responsible for early PMN activation since neutrophil exposure to LPS results in cell priming and/or
activation. However, plasma levels of LPS are not commonly elevated following thermal injury, and endotoxin cannot be confirmed as being responsible for early neutrophil activation. Various cytokines, colony-stimulating factors, and lipids such as platelet-activating factor, as well as leukotrienes, have been implicated in the priming or activation of neutrophils. Their relative importance, however, remains undefined.

CELL-CELL AND CELL-MATRIX INTERACTIONS

Three families of cell surface proteins orchestrate the interactions between leukocytes and endothelial cells that have been implicated in the pathogenesis of platelet aggregation, inflammation, ischemia-perfusion injury, endotoxemia, tissue repair, tumor invasion, and atherogenesis. Expression of the various cell surface receptors occurs following stimulation by endotoxin, the inflammatory cytokines interleukin (IL) 1 and tumor necrosis factor, thrombin, histamine, terminal complement components, and hydrogen peroxide as well as other oxygen radicals.

The three closely related selectins, E, P, and L, are cell surface glycoproteins that exhibit markedly different patterns of expression. E-selectin expression occurs solely on activated endothelial cells; P-selectin is stored in the storage/secretory granules of endothelial cells and platelets following constitutive synthesis by those cells; L-selectin is the only one constitutively expressed on the surface of neutrophils, lymphocytes, and monocytes from which it is rapidly shed. The selectins appear to mediate the early "slow rolling" phase of leukocyte emigration.

The integrins are another family of cell surface proteins that mediate the second phase of leukocyte transmigration, ie, the "tight adhesion" that occurs between leukocytes and vascular endothelial cells. The integrins formed by various subunit combinations possess variable adhesive substrate-binding capabilities. Lymphocyte function-associated antigen 1 is expressed on all white blood cells and mediates leukocyte attachment to endothelial cells. That attachment involves an interaction of the integrin with intercellular adhesion proteins such as intercellular adhesion molecule 1, a member of the immunoglobulin superfamily, is concentrated in the junctions between endothelial cells and expressed on the surfaces of both neutrophils and monocytes. It mediates the cell interactions essential for the endothelial transmigration of both monocytes and neutrophils distal to the "tight adhesion" process mediated by the integrins. Recent studies have shown that leukocyte emigration consists of three sequential and distinct steps—rolling, tight adhesion, and transmigration. Monoclonal antibodies and antagonists to the individual adhesion molecules and counter receptors are being evaluated for their effectiveness in interrupting that process in receptor-specific fashion.

ENDOGENOUS ORGAN INJURY

The CR3 (CD11a/CD18) mediated adherence of PMNs to microvascular endothelium is a crucial step in PMN-mediated injury. The association between increased CR3 expression and posttraumatic acute respiratory distress syndrome (ARDS) was recently described by Simms and D'Amico. Patients with clinical ARDS had increased CR3 expression on PMN cell surfaces, while patients with trauma without ARDS did not. In a subsequent study of 17 patients at risk for ARDS, CR3 expression increased prior to the clinical recognition of ARDS in six of the seven patients who subsequently developed this syndrome. CR3 expression remained normal in the other 10 patients who did not develop ARDS. These results suggest that posttraumatic ARDS is associated with an increased number of CR3 receptors on the PMN cell surface and subsequent upregulation of PMN function. The demonstration by Mileski and coworkers that monoclonal antibody 60.3, an antibody that recognizes the functional epitope on the B chain polypeptide portion of CD11a/CD18, decreased gastrointestinal injury in a primate model of hemorrhagic shock, lends further credence to the participation of PMN adherence in endogenously derived organ injury. Of particular importance is that the administration of this antibody did not increase mortality in a rabbit model of abdominal sepsis, indicating that the antibody does not interfere with the normal inflammatory response.

With the exception of a rat model of systemic complement activation using cobra venom factor, complement activation and subsequent upregulation of PMN function does not appear to be a sufficient stimulus to cause ARDS. Activation of endothelial cells within the lung by local inflammatory processes or a second systemic stimulus that further activates PMNs appears to be necessary for cell-mediated tissue injury.

The concept of specific priming and activation sequences in which a "second-hit" phenomenon is required for significant in vivo tissue injury was recently popular-
ized by Anderson and Harken. They demonstrated that in rats, low-dose LPS administration results in a priming of neutrophils that is partially mediated by platelet-activating factor. The subsequent exposure to a noninjurious dose of FMLP then results in significant pulmonary injury. Administration of the endotoxin or FMLP without prior LPS priming does not cause lung injury. The ability of low doses of FMLP to cause significant tissue injury following LPS is thought to be secondary to alterations in the N-formylpeptide receptor system. The receptors appear to be conformationally altered following priming (LPS-related expression) from a high-affinity to a low-affinity state in which ligand is released more readily, thus increasing stimulatory capacity. Such a hypothesis fits with our knowledge of FMLP receptor alterations that occur following complement activation in patients with trauma and thermal injuries. Although Anderson and Harken were able to block organ injury with platelet-activating factor antagonists, suggesting that the platelet-activating factor was at least partially responsible for neutrophil priming, other agents appear to participate in the process.

LYMPHOCYTE RESPONSE TO INJURY AND INFECTION

Injury also alters lymphocyte function in a dose-related fashion. The first evidence that the cell-mediated immune response was decreased in the massively traumatized patient was published over 40 years ago. Even so, whether decreased cell-mediated immune response is related to the increased susceptibility of the patient with trauma to infection remains enigmatic. Much of the suppression of the cell-mediated immune response is undoubtedly due to the increased corticosteroid and catecholamine levels present following severe trauma.

T-LYMPHOCYTE FUNCTION CHANGES AFTER INJURY

The assumption that patients with trauma are "immunosuppressed" has been called into question by the realization that many of the mononuclear cell preparations from these patients in earlier studies were seriously contaminated by nonlymphoid cells. Further, if the contaminating cells were removed by repurification of the Ficoll-Hypaque preparation, much of the "inhibition" of mitogen stimulation disappeared.

Our recent studies and those of others have clearly demonstrated that mononuclear cell preparations from burned patients contain cells that spontaneously take up tritiated thymidine (Table). Wound excision appeared to increase this spontaneous DNA synthesis. Cells from patients with burns and trauma have decreased ability to secrete IL-2 in vitro culture, and this defect apparently involves membrane receptors as well as the cytoplasmic cascade since the decreased IL-2 secretion could only be partially reversed by direct stimulation of the cytoplasmic components. Correspondingly, Quattrocchi et al reported that a subpopulation of cytotoxic cells requiring IL-2 for activation (lymphokine-activated killer cells) had decreased cytotoxic activity after severe head injury.

Using a mouse model of thermal injury, Deitch et al demonstrated that cellular environment influences activation, ie, mononuclear cells from several lymphoid tissues reacted differently to a variety of mitogenic stimuli after thermal injury. Using a similar mouse burn model, Moss et al found a correlation between decreased mitogenic response, IL-2 production, and increased susceptibility to mortality from peritonitis. Similarly, hemorrhage modified the secretion of IL-2 from mononuclear cells and decreased the ability of the cells to express IL-2 receptor after concanavalin A stimulation in a mouse shock model. A subpopulation of mouse cells expressing CD8 was shown to suppress the murine mixed lymphocyte reaction and interferon-γ secretion after thermal injury. This suppressor population was not directly correlated with susceptibility to infection or mortality.

B-LYMPHOCYTE FUNCTION CHANGES AFTER INJURY

In contrast to the many reports of suppressed functional capacity of T lymphocytes, the function of the antibody-producing B lymphocytes seems to be variably affected but in general less inhibited after injury. Spontaneous production of IgG in culture was reported to be increased (Table) or unchanged, while spontaneous production of IgM was reported to be decreased or not changed (Table). Pokeweed mitogen or Staphylococcus aureus Cowan 1 antigen–induced secretion of IgG were also variously reported to be increased or decreased. Reports of stimulated IgM synthesis also varied, being increased at some times after injury and decreased at others. In one study, in vitro addition of indomethacin restored pokeweed mitogen–induced IgM and IgG secretion to control levels.
Lymphocyte function is determined in large part by the antigens displayed on their surface. These surface antigens control cellular interactions and stimulate biochemical activities in the cytoplasm that determines the cell's functional response. Determination of mononuclear cell dysfunction cannot be complete until we understand the many surface molecular changes that occur after injury.

The resolving power of flow cytometry has permitted reliable and accurate definition of changes in lymphocyte subpopulations following injury. Since absolute numbers of lymphocytes decrease in the circulation following severe injury, all T-lymphocyte subpopulations decrease, the decrease in the proportion of CD4 lymphocytes being greater than that of CD8 within 48 hours and a larger proportion of CD8 cells being decreased for the following 3 weeks. The proportion of lymphocytes spontaneously expressing various activation antigens such as CD25 (IL-2 receptor), CD69 (activation inducer molecule), and CD71 (transferrin receptor) was increased 48 hours after thermal injury, in contrast to what had been reported previously for CD25. Proportions of cells displaying integrin receptors (CD11c, CD49a, and CD54) were also increased, but it was unclear whether these might have been due to granulocyte contamination since gradient purification was used to separate the mononuclear cells. The proportion of CD20 (B lymphocytes) and CD16 (natural killer cells) was unchanged while the proportion of CD14-positive cells (monocytes) was increased on day 1 and decreased on days 4 and 7 after thermal injury.

There also appears to be a decreased ability of CD4 and CD8 subpopulations to express CD25, HLA-DR, and CD71 in response to mitogen stimulation after thermal injury. Hoyt et al also demonstrated reduced CD25, CD71, and HLA-DR expression on mitogen-stimulated CD4 lymphocytes after head injury, but only a decrease in the expression of CD71 in mitogen-stimulated CD8 lymphocytes. Steroid administration to volunteers mimicked several of the subpopulation changes seen in trauma. Absolute T-lymphocyte populations decreased mostly because of a decrease in the proportion of CD4 lymphocytes while the proportion of CD20 and CD8 cells remained unchanged.

STUDIES IN animal models have revealed similar changes in lymphocyte populations following injury. Organ et al reported subpopulation changes in seven rat lymphoid compartments following a 50% total body surface burn. Each compartment had a unique pattern of subpopulation changes following burn injury. In general, every compartment except bone marrow exhibited a decrease in the proportion of T cells soon after injury. Blood and spleen had late increases in CD4-positive cells (12 days and 48 days after the burn, respectively) while the mesenteric lymph nodes and the nodes draining the wound had only decreased proportions of CD8-positive cells. Lα-positive cells increased in blood but remained relatively stable in all the other compartments except the thymus and the lymph nodes draining the wound which had increased proportions of Lα-positive cells later in the postburn period. Burleson et al, using a smaller burn (30%), reported a decrease in CD8 lymphocytes only in lymph nodes from burned rats. Only after infection was added to the injury was there a decrease in the proportion of both blood and lymph node T lymphocytes and no change in spleen T lymphocytes.

Hansbrough and Gadd extensively studied changes in mouse lymphocyte subpopulations following both burn and surgical trauma. In contrast to the rat and human, mouse blood and spleen CD4 and CD8 subpopulations decreased approximately 2 weeks after receiving the burn. Consis-
tent with this, Gough et al. showed no decrease in mouse splenic CD4 and CD8 subpopulations during the first week after the burn injury. The proportion of B lymphocytes decreased in spleen and blood but were unchanged in lymph node. The time course of the changes in T lymphocytes was shorter for surgical trauma and longer for burns. In contrast to the human studies, spontaneously activated CD4 and CD8 lymphocytes were decreased for both types of trauma. Burns, but not surgical trauma, caused splenic hypotrophy, which was related to the presence of burned tissue either on the wound or excised and placed in control animals. In agreement with the data from the human studies, mouse CD4 and CD8 subpopulations in thermal injury and hemorrhagic shock had a decreased ability to express the activation antigens IL-2 and la (comparable with HLA-DR in humans) after mitogen stimulation.

The significant technical problems that have made accurate measurement of lymphocyte phenotype and function impossible are gradually being overcome. New techniques in flow cytometry make it possible to measure whole blood samples and eliminate the need for lymphocyte separation. Procedures have also been developed to correct for debris and nonlymphoid cells that may contaminate the light scatter gates in whole blood preparations from patients with severe trauma and to decrease the dependence on nonspecific light scatter techniques to eliminate the contributions from nonlymphoid cells. These new techniques should improve the accuracy and specificity of measurement of surface antigens in injured patients and make possible a reliable assessment of their clinical relevance and importance.

CONCLUSIONS

We now recognize that following injury, leukocyte populations are strikingly changed and that leukocyte function is altered in a more complex fashion than simple hypofunction. Continuing advancement and understanding of the molecular biologic mechanisms that regulate the inflammatory response will allow us to clarify the relationship between initial injury, leukocyte abnormalities, and subsequent clinical disease. No unifying hypothesis for the cause of the susceptibility of patients with trauma to infection has emerged to replace the theory of global immunosuppression. It is clear that there are marked changes in leukocyte function and phenotype in response to injury, but the relationship of these changes to infection remains to be clarified. Since the leukocyte response to injury is not an isolated response but one that is integrated with the total physiologic response to injury, specific pathogenetic mechanisms have been difficult to define. As our understanding of the deleterious and beneficial parts of this response expands, we will be able to devise treatment strategies to optimize the beneficial aspects of the responses while minimizing or even preventing the damage caused by the potentially deleterious responses.

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