The Immunologic Response to Injury

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Research on the immune consequences of shock and trauma by multiple laboratories over more than 20 years has resulted in the following paradigm, which is currently accepted by most investigators in this field: serious traumatic or thermal injury is quickly followed, after initial resuscitation, by the systemic inflammatory response syndrome (SIRS) which, in a sizeable minority of patients, will lead inexorably to multiple organ dysfunction syndrome (early MODS), with an attendant high mortality. The majority of seriously injured patients survive the initial SIRS response without developing early MODS, and after a period of relative clinical stability, manifest a compensatory antiinflammatory response syndrome (CARS) with suppressed immunity and diminished resistance to infection. Resultant infection and its attendant inflammation in turn can lead to multiple organ dysfunction (late MODS) and death (Fig. 1).

This paradigm has several implications of potential importance in interpreting the sometimes conflicting results of research in this area:

1. An investigator's view of the immune consequences of serious injury may depend not only on what is being measured but on when the measurements are made. Serial observations are mandatory.
2. The inflammatory SIRS response, which occurs immediately after injury, is very unlikely to be caused by sepsis; an inflammatory response that follows the CARS syndrome is very likely to be induced by invasive infection.
3. CARS is most probably the direct result of earlier SIRS.
4. Because treatment of early and late MODS is largely supportive, it is reasonable to suppose that therapies directed at modulating SIRS or blocking CARS, preventing the onset of MODS, will prove to have more practical benefit to injured patients than efforts to treat MODS once it has ensued.

In the past most investigators have tended to attribute postinjury SIRS to hyperactivity of the innate immune system[1-4] and CARS to dysfunction of the adaptive immune system,[5-9] but recent evidence[10-12] supports the logical conclusion that interactions between the innate and adaptive immune systems are important in the induction of both SIRS and CARS. For example, evidence has accumulated that the SIRS response, which quickly follows major injury, may be a manifestation of the ischemia and reperfusion syndrome, well studied in a number of animal models. Certainly almost every seriously injured patient has undergone a period of diminished circulating blood volume with accompanying hypotension. Resuscitation would be likely to trigger the abnormalities known to occur with reperfusion of ischemic tissue. Under most circumstances, circulatory autoregulation would dictate a more severe hypoperfusion of the gut than of most other organs during the period of hypovolemia. Ischemia and reperfusion of the gut in particular has been shown experimentally to induce considerable local and remote organ damage.[13-15]

Although it is established that activated neutrophils (PMN) and macrophages and their products, such as reactive oxygen species (ROI), nitric oxide (NO), and eicosanoids play an important role in tissue injury after ischemia and reperfusion,[15-19] experiments using genetically altered animals have demonstrated that tissue damage from ischemia and reperfusion is critically dependent on activation of complement through the classical pathway mediated by natural IgM antibody produced by B1 lymphocytes.[20-22] In animals lacking one of the complement proteins necessary for classical pathway activation, or who are depleted of IgM antibody, little, if any, tissue damage occurs after ischemia and reperfusion. Surprisingly, the antigen (s), presumably widely expressed by ischemic endothelium, which binds IgM and activates complement, has yet to be identified. Nevertheless, this work strongly suggests that B lymphocytes from the adaptive immune system play a vital role along

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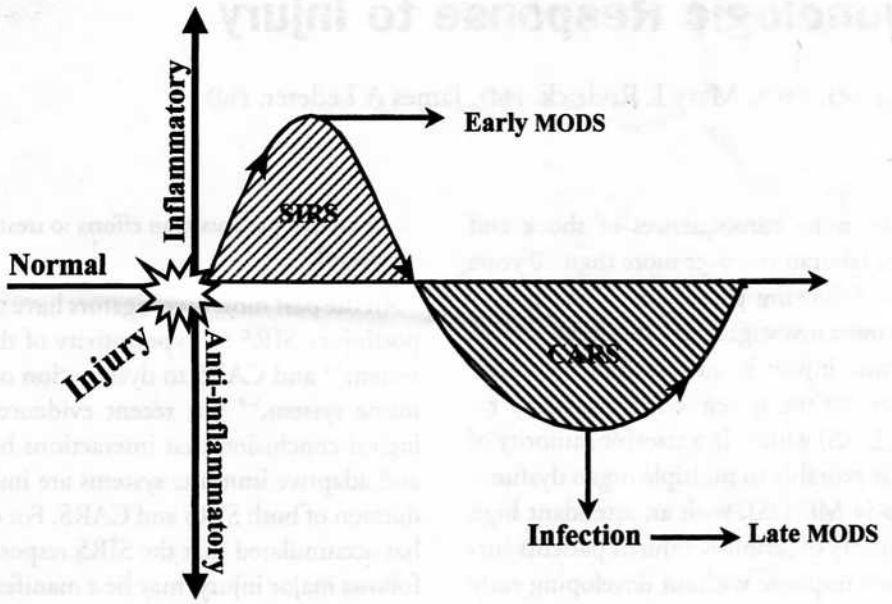

Figure 1. Serious injury is immediately followed by a systemic inflammatory response (SIRS), which in a minority of individuals (20% to 30%) is persistent and may lead to the multiple organ dysfunction syndrome (MODS) with an attendant high mortality rate. In the majority of individuals the SIRS response resolves without leading to early MODS and is then followed by a compensatory antiinflammatory response (CARS) associated with diminished resistance to nosocomial infection. Infection can again induce systemic inflammation leading to late MODS. Like early MODS, late MODS results in substantial mortality. (Adapted from Moore FA, Sauaia A, Moore EE, et al. J Trauma, Injury, Infection, and Critical Care 1996;40:501–512 with permission.)

with components of the innate immune system in initiating endothelial and parenchymal damage after resuscitation from shock induced by injury.

It now seems very likely that T cells from the adaptive immune system play a role in the early SIRS response to injury. For example, we have used T-cell receptor transgenic mice and bacterial superantigens in wild type mice to demonstrate that naïve T cells are primed immediately after injury to produce large and sometimes fatal quantities of interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) on stimulation of their receptors (TCR) by antigen.[10,11] IFNγ is the classical "macrophage activating factor" and is an excellent inducer of proinflammatory cytokine and NO production by cells of the innate immune system. The mechanisms responsible for priming T cells for an exaggerated proinflammatory response when their TCRs are presented an appropriate antigen early after injury are unknown. But likely candidates are the costimulatory cytokines interleukin-1 beta (IL-1β) and interleukin-12 (IL-12), known to be produced in increased quantities early after injury[1,21,22] by antigen presenting cells (APC) of the innate immune system, and the interactions of costimulatory molecules on T cells with their ligands on APC. The antigen (s) responsible for T-cell activation early after injury, like those responsible for complement activation after reperfusion, remain undefined. Possibilities include intracellular "neoantigens" or superantigens, such as mouse mls, which may be expressed or released by injured or necrotic cells and presented to T cells with the appropriate TCR, causing clonal expansion followed later by assumption of a suppressive or regulatory phenotype, which is a common sequela of intense antigenic stimulation.[23-30]

Though it now seems clear that the adaptive immune system contributes to postinjury SIRS, the syndrome is clearly characterized by the release of proinflammatory cytokines, eicosanoids, and other inflammatory mediators such as ROI and NO by cells of the innate immune system.[1,4,15] But the mechanisms, other than IFNγ, triggering the release of inflammatory mediators after hypovolemic shock or reperfusion injury are incompletely understood. Recent clinical and animal research has cast doubt on the role of endotoxin leaked from the gut in this regard.[1,4] But other undefined molecules contained in gut lymph from animals subjected to hemorrhagic shock can initiate systemic inflammation.[31,32]

The role of the products of complement activation in

inducing production of proinflammatory mediators by cells of the innate immune system also remains cloudy. There is evidence that the products of complement activation. C5a, C5a desarg, or both, are important in the induction of local TNFα production in an animal model of immune complex induced lung injury.[33] Other carefully done in vitro studies suggest that the products of complement activation including C5a are not sufficient to induce proinflammatory cytokine production by monocyte/macrophages,[34,35] but may act in synergy with endotoxin or IFNγ to do so.[34] But in an animal model of burn injury, it was found that splenic monocytes and macrophages were primed by injury to produce supranormal amounts of proinflammatory cytokines in animals genetically deficient in C3, the pivotal molecule in both classical and alternative pathways of complement activation.[36] On the other hand C5a is a strong activator of PMN and monocytes and a chemoattractant, and it may play an important role in priming these cells by upregulating receptors involved in their recruitment to local areas of injury and to remote organs where they contribute to tissue damage.[37,38] The proinflammatory cytokines IL-1β and TNFα also facilitate this process by increasing expression of adhesion molecules by endothelial cells, promoting adherence and eventual transmigration of activated leukocytes.[1,39]

Injured tissue itself may play a major role in initiating the SIRS response. Recent work from Matzinger's laboratory has shown that necrotic, but not apoptotic, parenchymal cells will activate cells of the innate immune system sufficiently to induce a productive response by the adaptive immune system.[40] The molecular mechanisms involved were not explored by these investigators. Recent reports from other laboratories suggest that at least one class of intracellular proteins, the heat shock proteins, strongly activate the innate immune system when they are released into the extracellular environment.[41,42] In fact one such heat shock protein, HSP 60, has been shown to be a natural ligand for toll-like receptor 4, the signaling receptor for endotoxin, expressed on cells of the innate immune system.[43]

In summary, the SIRS syndrome, which regularly occurs after serious injury, and in some cases proves fatal to the injured individual, has been partially characterized by both clinical and animal research with respect to its pathologic, biochemical, and immunologic manifestations. But, the triggering mechanisms remain incompletely defined, as are the patterns of gene expression and the signaling systems involved in inducing and maintaining the SIRS response.

Attempts at clinical therapies for SIRS have included resuscitation with hypertonic saline, which appears to have the unexpected advantage of being antiinflammatory,[44,45] and the use of cyclooxygenase inhibitors[1,46] and corticosteroids.[47] In addition, treatment of SIRS in animal models of hemorrhagic shock and ischemia and reperfusion has included soluble complement receptors,[20] interleukin-10 (IL-10),[48] antibodies directed at inflammatory cytokines,[49] cytokine receptor inhibitors,[1] superoxide scavengers,[50] phosphodisterase inhibitors,[13,51] antiadhesion molecule antibodies,[39] NO scavengers,[15] NO synthase inhibitors,[16,18] or conversely, NO donors,[52] among others. Some of these agents are undergoing clinical trials.

The CARS syndrome, which has only recently acquired its acronym,[53] has been postulated to occur in seriously injured patients for many years based on anecdotal clinical observations suggesting that such patients were more prone to become infected with nosocomial organisms than normal individuals or hospitalized patients with conditions other than major injury. These clinical impressions were reinforced by observations that circulating T lymphocytes taken from critically injured patients several days after injury failed to proliferate to mitogenic stimuli[54,55] and also failed to produce the stimulatory cytokine, interleukin-2 (IL-2).[5] The degree of suppression of IL-2 production could also be correlated with the likelihood of the subsequent development of sepsis, though a causal relationship was not established.[5] Further clinical observations demonstrated that several days after serious injury, patient T lymphocytes also demonstrated diminished capacity to produce IFNγ on polyclonal stimulation.[6,8] Subsequent studies by several groups indicated that diminished IL-2 and IFNγ production by injured patients' T cells was frequently accompanied by increased production of IL-4 and IL-10.[56-58] These findings suggested, but did not prove, that circulating T lymphocytes in these individuals had undergone a phenotypic shift from proinflammatory type 1 cytokine production to production of inhibitory type 2 cytokines. Increased IL-10 production by circulating T cells and increased IL-10 levels in plasma within the first 10 days after injury were also associated with an increased incidence of subsequent sepsis in our studies[56] and those of Sherry and associates.[57]

To understand the CARS syndrome more clearly several groups of investigators have turned to animal models that appear to manifest CARS after injury.[7,8,58] These include rodent models of burn injury used by our laboratory[59] and others, and of hemorrhage combined with soft tissue or bony trauma used by Wichmann and associates.[60] Both of these injury models support clinical impressions of increased susceptibility to infection in seriously injured patients in that the animals subjected to injury show a marked increase in mortality to a septic challenge in the form of cecal ligation and puncture (CLP) at predictable times after injury[58,59] when compared with sham injured controls. The organisms involved are among those commonly encountered in sepsis associated with the CARS syndrome in injured patients. So these models have been used to study the kinetics of the CARS syndrome and to establish the relationship of suppressive cytokine production to diminished resistance to infection. In both models increased mortality from CLP is associated with increased production of IL-10 and diminished production of IL-2 and IFNγ.[58-60,78]

So the CARS syndrome, both clinically and in these animal models, appears to be associated with diminished production of type 1 and increased production of type 2 cytokines by T cells. But there has been considerable disagreement as to whether injury actually induces a phenotypic shift from predominance of type 1 T helper cell function to that of type 2 T helper cells.[8,56] In the mouse burn model we have addressed this question by immunizing both burn and sham burn animals at the time of injury and have demonstrated that burn but not sham burn injury led to loss of production of the T helper 1-dependent antibody isotype IgG2a, with normal or increased production of the T helper 2 antibody isotypes IgG1 and IgE.[61,62] These studies clearly demonstrated that, in the mouse, burn injury is followed by loss of T helper 1 function in vivo with preservation of T helper 2 function. Although these events are temporally related to lowered resistance to infection, they do not prove a causal relationship.

But some light on the cause of the CARS syndrome has been shed by recent experiments in mouse models of burn injury and of trauma and hemorrhage. In both models diminished resistance to infectious challenge in the form of CLP can be prevented by administration of anti-IL-10 monoclonal antibody early after injury.[63,64] These experiments indicate that in injured animals the early production of IL-10 is sufficient to induce the CARS syndrome and increased susceptibility to infection. On the other hand, we have also demonstrated that in IL-10 knockout mice burn injury, nevertheless, induces loss of T helper 1 function with respect to antibody isotype production, and markedly diminished antigen specific IFNγ production.[65] So, in the absence of early IL-10 production, other mechanisms can compensate to induce a CARS-like state.

Other possible mediators contributing to the CARS syndrome include prostaglandins of the E series, which are known to be produced by cells of the innate immune system in response to injury,[46] and corticosteroids, which are released into the circulating plasma at the time of injury and for a variable period of time thereafter.[1] Both of these mediators are known to diminish production of IL-2 and IFNγ by cells of the adaptive immune system while maintaining or increasing the production of the suppressive mediators IL-4 and IL-10.[66,67] Nitric oxide, which is often produced in increased quantities after injury, is known to inhibit IFNγ synthesis.[68] The production of transforming growth factor beta (TGFβ) also has been reported to be increased after injury.[69] This cytokine can inhibit activation of both the innate and adaptive immune systems.

In addition to anti-IL-10 antibody, other therapies effective in reducing septic mortality in animal models of injury include IL-2, IFNγ;[70] anti-IL-6 antibody,[71] melantonin,[72] prolactin,[73] granulocyte macrophage colony stimulating factor (GM-CSF),[74] the testosterone receptor antagonist, flutamide,[75] and IL-12, the cytokine that induces the T helper 1 phenotype.[76,77] IL-12 production in response to endotoxin stimulation is reduced in circulating peripheral blood mononuclear cells from seriously injured patients and in adherent cells from the spleens of burn but not sham burn mice, beginning several days after injury and reaching a nadir at 7 to 10 days, the time of maximal production of IL-10.[77]

Unfortunately, neither of the two biologic agents, anti-IL-10 antibody or IL-12, which we have found most effective in restoring normal resistance to CLP in the mouse burn model, appears to be ideal for prophylactic treatment of injured patients to prevent CARS and subsequent sepsis. Anti-IL-10 antibody has been shown to be detrimental to survival when administered after CLP in mice,[3] and IL-12 therapy, when used clinically in the treatment of patients with cancer, has shown considerable toxicity, very likely caused by increased IFNγ production.[78]

Most clinical and animal studies suggest that the CARS syndrome, in keeping with its name, is truly a compensatory response because it does not appear to occur either in injured patients or relevant animal models without earlier SIRS. So it is likely to be a carefully conserved, genetically programmed, response to inflammation. Recent experiments in our laboratory indicate that development of the suppressive T-cell phenotype after injury, which appears to be a hallmark of CARS, does not occur without the activation of T cells through the recognition by their TCR of antigen presented by APC early after injury. The use of T-cell transgenic mice has shown that naïve T cells taken from these animals as long as 7 days after burn injury are primed to make an exaggerated proinflammatory, IFNγ response when presented with the antigen to which they are genetically programmed to react.[10] But if small amounts of the same antigen are administered in vivo at the time of injury, T cells harvested a week later produce almost no IFNγ on presentation of that antigen, but instead secrete large quantities of the suppressive cytokines IL-4 and IL-10.[79]

These results have also been substantiated in wild type animals through the use of superantigens, which, when presented by APC, activate the TCR of approximately 20% of T helper cells, as a means to reveal the postinjury T-cell phenotype. These studies have shown that T cells early after injury are programmed to respond to superantigen stimulation with markedly increased production of IFNγ and TNFα when compared with T cells from sham injured mice.[11] But by a week after injury T cells from burn injured animals produce increased quantities of IL-10 in response to superantigen presentation and diminished quantities of IFNγ and TNFα when compared with sham burn controls.

In summary, although IL-10 has emerged as an important mediator of the CARS syndrome in injured patients and animals, the molecular mechanisms involved in triggering its production are poorly understood. Although several biologic agents have shown some efficacy in preventing the lowered resistance to infection characteristic of the CARS syndrome in animal models, and IFNγ has, in fact, been used clinically for the same purpose in injured patients with equivocal results,[53,80] many investigators agree that a safe and effective agent for the prophylactic treatment of injured patients to prevent CARS has yet to be identified.

It is evident that MODS is the principal cause of death after serious injury in patients who survive initial resuscitation. In a sizable minority of such patients the initial SIRS response to injury leads inexorably to MODS; in the majority of individuals who die from MODS, the syndrome follows the onset of sepsis associated with the antiinflammatory CARS response. There is, at best, a rudimentary understanding of the pathologic and molecular processes involved in the progression from the inflammation of SIRS or sepsis to damage in multiple organ systems and death from MODS. Progress in this area has been impeded by the lack of relevant animal models of MODS. Perhaps the one easily reproducible model that appears to have the clinical features of early MODS is that induced by the injection of zymosan into rodents.[81] At lower doses zymosan causes a prompt, systemic inflammatory response that is not immediately fatal and can induce later and pathologically demonstrable organ damage in the lungs, liver, and kidneys of the treated animals,[82] which, in turn, is associated with an appreciable late mortality. Zymosan, which was the agent used to define the alternative pathway of complement activation, is also a powerful activator of cells of the innate immune system. Experiments with the zymosan model have demonstrated diminished mortality in animals depleted of macrophages.[83] But in animal models of hemorrhagic shock with subsequent inflammatory responses resembling human postinjury SIRS or reperfusion injury, much of the tissue damage appears to result from mediators released from infiltrating PMN.[1,4,39,84] Whether early MODS is, in fact, simply the cumulative result of tissue injury from infiltrating inflammatory cells and the complement membrane attack complex remains speculative.

As yet there is no relevant animal model for late MODS that follows CARS-associated sepsis. Although it is tempting to speculate that the inflammation associated with persistent sepsis damages organ systems in a manner similar to that associated with hemorrhagic shock and reperfusion injury, this idea remains unproved. Certainly prevention of MODS by effective therapy for SIRS and CARS will likely prove more beneficial to injured patients than advances in therapy for this perplexing syndrome.

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