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Review

Immune Balance in Critically Ill Patients

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Abstract. The systemic inflammatory response reflects the non-specific clinical expression of a profound activation of the body's immune responsive elements. Immune activation and immune suppression coexist in the blood of patients with severe sepsis. It is their interaction and the resultant host parenchymal responses that ultimately define the course of sepsis. Importantly, neither profound immune activation (pro-inflammatory) or immune suppression (anti-inflammatory) characterize the dominant process. Rather, there is a combined low grade pro--inflammatory state associated with an immune hyporesponsiveness that defines the usual immunologic state of the patient with severe sepsis.

Key words: inflammatory response; PMN leukocyte activation; inflammatory mediators.

Introduction

Although the systemic inflammatory response syndrome (SIRS) has been well described[13, 17] on gross clinical terms, its actual cellular components and their interactions have only just recently come into focus. The overall inflammatory state and subsequent organ injury occurs as a result of inflammatory mechanisms. Many of the organ-system injury pathways are linked through immune effector cell activation and cytotoxic cell injury. The understanding of the processes that lead to immune injury and external modulation of the endogenous control of these immune effector cells during SIRS has proven to be complex. No simple cascade of initiating sequences followed by activation of intracellular inflammatory responses and subsequent cell and organ injury explains the process seen clinically, although it does describe the initial aspects of isolated cell culture experiments. Clinically, multiple pro- and anti-inflammatory mediators appear in the blood of patients with SIRS. Most of the time when one measures the levels of inflammatory mediators in the blood of patients with SIRS, the concentration of anti-inflammatory mediators vastly outweighs that of pro-inflammatory mediators[8]. Furthermore, it has been difficult to demonstrate bioactivity of the pro-inflammatory substances in the plasma of patients with SIRS[2]. Potentially, bursts of inflammatory mediator release are very brief or pro-inflammatory mediator molecules are bound to cell surfaces and are presented locally at high concentrations cell to cell[5]. However, based on measures of circulating pro- or anti-inflammatory mediators in the serum, it is unclear under what circumstances biologically relevant inflammation exists in the vascular compartment. This reality has led investigators to explore alternative methods of assessing the balance between pro- and anti-inflammatory mediators on a functional level. One of the means of assessing these complex interactions is to measure immune responsiveness of circulating immune effector cells.

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Inflammatory Balance in SIRS

One important potential consequence of pro-inflammatory activity in the blood is the activation of circulating polymorphonuclear neutrophil leukocytes (PMN). Activated PMN can bind to already injured endothelial surface cells. They have increased oxidative burst activity and are limited in natural apoptosis, thus insuring that their inflammatory activity will be prolonged. In this regard, PMN have been implicated in the organ dysfunction of SIRS. We[15] and others[11] have shown that circulating PMN from patients with SIRS appear partially activated with a higher than normal display of CD11b, a β2-integrin adhesion molecule that is increased in density on the cell surface upon PMN activation. CD11b combines with CD18 to form the integrin adhesion molecule necessary for firm adhesion of PMN to activated endothelial cells. The display of adhesion molecules on circulating PMN can be used to identify a potentially activated phenotype. With pro-inflammatory stimulation PMN display increased CD11b and diminished L-selectin.

CD11b is a good marker of PMN activation because it is a functional molecule that is involved in many of the potent inflammatory mechanisms of the PMN. CD11b is up regulated within minutes of exposure to pro-inflammatory substances such as TNF-α, IL-8, formyl-methionyl-leucyl-phenylalanine, C5a, leukotriene B4, and platelet activating factor. However, like other integrins, CD11b must not only increase in density on the cell to work, but it must undergo a qualitative change as well. When PMN activate, intracellular signals alter the conformation of CD11b to a new 3-dimensional structure that can bind its target receptors. Activated CD11b can be recognized by antibodies that bind to "activation neoepitopes" present only on active CD11b[6] referred to as activated CD11b. Binding of antibodies to activated CD11b blocks adhesion to counter-receptors. Only activated CD11b binds to the vascular endothelium via intracellular adhesion molecule-1 (ICAM-1) and to complement component iC3b, clotting factor X, fibrinogen and collagen type I. Furthermore, engagement of active CD11b with counter-receptors sends a signal into the cell markedly enhancing the respiratory burst[18]. Thus, the activation of CD11b plays a role in attachment to endothelium, phagocytosis of bacteria, enhancing the respiratory burst, bacterial killing, interaction with clotting components and migration of PMN within tissues. Many of the potent inflammatory functions of the PMN require the activation of CD11b, and active CD11b defines PMN engaged in inflammatory behavior.

The circulating PMN of patients with SIRS are constantly exposed to a complex mix of inflammatory mediators. They have a higher density of CD11b at rest than do PMN from normal controls. We hypothesized that these PMN are partially activated. The level of activation, however, can not be assessed by merely measuring the PMN expression of CD11b or its activated epitope, because these markers only denote what has happened, not what can subsequently occur in response to an immunological challenge. If these PMN from patients with SIRS have a greater potential for further activation of CD11b this would lead to greater PMN adhesiveness and a more vigorous respiratory burst. These events may increase endothelial and tissue injury. Thus, assessing the ability of these cells to increase further their expression of CD11b would characterize the relative balance between pro- and anti-inflammatory stimuli as they interact in a complex fashion with circulating PMN. If these partially activated PMN could be demonstrated to further activate their phenotype in response pro-inflammatory stimuli, then this situation would provide a rationale for the use of anti-inflammatory therapies, including the use of monoclonal antibodies against CD11b in SIRS. However, if these PMN had a blunted activation response to pro-inflammatory stimuli, as compared to normal controls, then these data would demonstrate a relative immuno-suppression of circulating PMN, arguing more for immuno-stimulating therapies, if any form of immunomodulation were to be undertaken at all.

Accordingly, ROSENBLOOM et al.[16] measured the quantitative and qualitative changes in CD11b that occur during PMN activation *in vitro*. They stimulated freshly isolated whole blood PMN by adding increasing doses of TNF-α (0–200 U/ml) for 15 min prior to fixation for flow cytometry. Thirty-six patients who fulfilled the SIRS criteria[1] and also had a known source of serious infection, including peritonitis, intra-abdominal or intra-thoracic abscess, pneumonia, upper urinary tract infection with obstruction and perforated abdominal viscous were selected. The essence of this study was to describe both the *in vitro* expression of CD11b and its active form on circulating PMN and their subsequent immediate response to varying levels of TNF-α. Thus, the immediate *in vitro* expression of CD11b was used as a measure of the *in vivo* responsiveness or immune competence of our subjects. The goal of this assay is to document immune competence the immediate response of circulating PMN to a pro-inflammatory stimulant in terms of their basal and subsequent response to TNF-α.

Results

Not surprisingly, the patients with SIRS had elevated levels of pro-inflammatory cytokines IL-6, IL-8 and TNF-α compared to normal controls. However, the levels of antagonists of inflammation were much higher patients with SIRS. Soluble TNF-RII but not soluble TNF-RI correlated inversely to organ failure in the patients. While neither correlated with mortality or severity of illness scores. Similarly, the patients with SIRS had higher CD11b levels and lower L-selectin levels than controls.

The normal response of CD11b on PMN upon exposure to TNF-α appears to be a rapid up-regulation of the density of total CD11b, with a simultaneous increase of the density of CBRM1/5 staining. These changes indicate a conformational change in CD11b, which is associated with activation and receptor binding capability. Although SIRS patients start out with a slightly higher average CD11b display, as PMN are exposed to TNF-α *in vitro*, the density of CD11b on healthy PMN challenged with TNF-α surpasses that on the patients cells as the amount of TNF-α used is increased. The amount of CD11b displaying the activation epitope reactive with CBRM1/5 followed a similar pattern. No cytokine level measured in the blood correlated significantly with the diminished responsiveness of CD11b.

The suppression of CD11b responsiveness to TNF-α on PMN is most likely of multi-factorial etiology. The functional defect appears to be within the cells themselves, since there was no correlation with levels of soluble TNF receptors and no relationship to TNF receptor loss from the PMN. Also, CD11b dysfunction did not appear to be due to cell immaturity. There was no correlation with the number of circulating immature forms. Potentially, exhaustion of intracellular stores of CD11b[19] or senescence of PMN due to inhibition of apoptosis[3] could induce these phenomena. Whatever the causes of diminished CD11b responsiveness, this dysfunction appears to affect all circulating PMN.

The magnitude of CD11b unresponsiveness in the patients with SIRS correlated with the degree of organ failure. However, SIRS criteria are grossly non-specific and up to 2/3 of all hospitalized patients fulfill the SIRS criteria. This implies that diminished CD11b function is widespread in critically ill patients, and may be a non-specific effect of critical illness. In support of this hypothesis, a diminished quantitative CD11b up--regulation in response to mediators of inflammation has also been shown in burn patients[14]. In fact, these phenomena probably reflect the broader immune response referred to as inflammatory-stimuli-induced-anergy as proposed by Cavillion.

Does immune responsiveness reflect process or outcome from acute illness? More than likely, outcome from SIRS is a complex endpoint. In the ROSENBLOOM et al. study[16], there was no relationship between the magnitude of CD11b dysfunction and mortality. Presently, we favor the hypothesis that immune suppression is the natural process of inflammation activation. The diminished immune responsiveness of circulating PMN in infected, critically ill patients probably reflects the natural down regulation of the inflammatory response which initially induces a pro-inflammatory response, including migration of PMN to the site of inflammation, followed by a down regulation of this response as repair occurs. This is an analogous process to thrombolysis following thrombus formation. Thus, immune suppression may be the method by which localized inflammation balances the risks of PMN over-stimulation and remote tissue injury versus under-stimulation and diminished clearance of infection. In support of this hypothesis, the relationship between CD11b function and both inflammation and infection and closely linked in a reciprocal fashion. Blockade of CD11b with monoclonal antibodies diminishes inflammatory injury is animal models[12]. The oxicam class of anti-inflammatory drugs antagonizes the appearance of the activated CD11b neoepitopes and this is thought to be one of the mechanisms of action. Conversely, CD11b blockade[4] or congenital absence of CD11b[7] increases the severity of infection.

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