The chronic consequences of severe sepsis

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Abstract: The early events of severe sepsis set in motion a cascade of events that significantly contributes to the morbidity and mortality observed during the first few days of this syndrome. Although sepsis is a deadly, acute disease, survivors also suffer long-term consequences. Clinical data underscore subsequent high mortality rates associated with patients who are long-term survivors of the acute septic episode. Within 1 year of surviving severe sepsis, there is a 26% predicted mortality rate, and many patients succumb to lung complications. In this review, we focus on the cellular and molecular mechanisms that dictate the longer-term sequela of sepsis and related lung injury. We have established a murine model of experimental sepsis [cecal ligation and puncture (CLP)], which results in an approximate 60% survival rate. Our studies have demonstrated that these survivors are susceptible to a fungal infection with 100% mortality when challenged 3 days or 15 days post-recovery from the initial CLP. This increased mortality correlates with changes in cytokines and Toll-like receptor expression and alterations in lung leukocyte populations. We hypothesize that the lung becomes predisposed to nosocomial infections for extended periods of time after severe sepsis via mechanisms that include alterations in inflammatory cytokines and an increase in immunomodulatory chemokines, such as monocyte chemoattractant protein-1 and C10. These mediators may alter the innate-immune response by affecting dendritic cells and macrophages, which could provide a mechanism for the immunosuppression observed following sepsis. *J. Leukoc. Biol.* 75: 408–412; 2004.

Key Words: *innate immunity · acquired immunity · long-term · lung · chemokines · Toll-like receptors*

INTRODUCTION

General consensus supports the idea that the acute inflammatory response is intimately linked to innate immunity and occurs over a relatively restricted chronological period. This traditional notion of the limited time frame of a functional acute inflammatory response has historically been supported by the temporal expression of a set of inflammatory mediators and leukocyte subpopulations. For example, within minutes of in vivo lipopolysaccharide (LPS) challenge to the peritoneum, mRNA expression for early response cytokines, such as tumor necrosis factor α (TNF-α), can be detected in mononuclear phagocytic cells followed by the expression of more distal cytokines and chemokines. This is followed by the recruitment of polymorphonuclear cells, which occurs within hours. Over the subsequent 24- to 48-h period, cytokine, chemokine, and leukocyte levels will subside, and any alteration in local tissue will be repaired [1, 2].

It is interesting that this scenario is dependent on the quantity (amount) and quality (complexity of the challenge composition) of the initial antigen or pathogen. An increase in the amount of the challenge will result in a heightened, acute cytokine and leukocyte response, and an increase in the complexity may lead to a prolonged response. An additional factor that determines the course of an acute inflammatory response is the severity of the host's response to the inciting agent. This aspect of the response has important consequences for the short-term course of the acute reaction but also appears to play a key role regarding the host response to a subsequent challenge that occurs long after the initial severe acute reaction. An interesting correlation exists between the severity of the initial, acute inflammatory response and the ability of the host to deal with a longer term, chronic challenge [3, 4].

One of the best examples of the chronic effects of severe acute inflammation is presented by data modeled from human septic populations. A variety of investigations have studied the short-term (less than 30 days) sequelae of the septic response and have reported mortalities of 30–50% [5]. Findings such as these have resulted in labeling this syndrome as a deadly, acute disease. However, additional investigations have noted that the septic population is at a significant risk of dying of nonseptic causes for up to 8 years after the initial hospitalization [3, 4]. Thus, the initial, severe response sets in motion a dysregulated, immune/inflammatory response, which has long-lasting implications regarding how the host can respond to and deal with other challenges. The chronic problems of severe acute inflammation are not limited to disorders such as sepsis but are found in a wide array of human diseases, including the long-term consequences of ischemia/reperfusion injury post-organ transplant [6], the alterations post-recovery from severe respiratory syncycial virus (RSV) [7, 8], and the changes found in burn and trauma injury [9, 10] (**Table 1**). This review will focus on the chronicity of severe acute inflammatory responses.

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TABLE 1. Chronic Complications Associated with Severe Acute Inflammatory Responses

- Chronic and life-threatening complications after surviving severe sepsis [3, 4]
- Chronic transplant rejection associated with initial severe ischemia/reperfusion injury [6]
- Chronic asthma increases after severe acute inflammation induced by respiratory syncial virus [7, 8]
- Chronic problems accompanying survival of severe acute inflammation caused by severe burns [9, 10]

THE CHRONICITY OF SEVERE ACTUE INFLAMMATION AND SUBSEQUENT CLINICAL COMPLICATIONS

The above clinical data support the view that a number of long-term consequences are associated with surviving severe sepsis. It is interesting that this is not the only clinical example of acute inflammation subsequently interphasing with chronic inflammatory disorders. As shown in Table 1, there are a number of clinical instances whereby an initial severe acute inflammatory event has been associated with distal chronic complications. Pediatricians who deal with severe acute RSV infections in very young children are clearly mindful of the longer term but ill-understood clinical complications, which these children have in dealing with severe chronic asthma. Transplant surgeons are aware of the problems that place a solid organ transplant at long-term risk if during the initial surgery, the donor organ has severe acute ischemia reperfusion injury. Burn and trauma physicians are cognizant of the patients who recover from the acute and severe inflammatory events of burn injury yet are susceptible to chronic clinical complications later in life. One of the uniting themes of the above clinical examples is that there appears to be a correlation with the severity of the initial acute inflammatory event that caused alterations in the innate and/or acquired immune system, which is manifested for not only months but often years after the initial severe insult. The mechanism(s) that are responsible for the chronic consequences of severe acute inflammation are presently not well understood; however, incriminating factors likely include alterations in the normal levels and activity of chemokines, cytokines, Toll-like receptors (TLRs), and dendritic cells (DCs).

CHEMOKINES IN CLINICAL AND EXPERIMENTAL SEPSIS

Over the past decade, a number of chemokines have been identified that possess interesting biological activities in innate and acquired immune response. Although chemokines clearly play a crucial role in the initiation and maintenance of inflammation via leukocyte recruitment, these protein mediators are involved in other immune-related processes, such as angiogenesis, cell activation, healing, repair, and end-stage fibrosis [11]. Chemokines have been divided into four subfamilies based on their unique sequence homology and the position of cysteine residues in the protein; the CC and CXC chemokine families comprise the largest groups with diverse activities [11]. Elevations in numerous CXC and CC chemokines have been detected in clinical diseases, such as asthma, pneumonia, colitis, central nervous system infection, gastritis, and sepsis. These clinical studies have provided the impetus to study the role of chemokines in established animal models of human diseases, including sepsis [12].

In this review, we focus on the putative role of chemokines in the pulmonary immunosuppression following experimental sepsis. Investigations into the long-term consequence of sepsis in survivors, especially with regard to the fate of immune activity of the lung, have been lacking, although clinical studies have demonstrated considerable morbidity and mortality associated with these individuals. It is noteworthy that a number of clinical studies support the observation that the long-term morbidity and mortality of sepsis patients are dependent on the severity of the initial insult [3, 4]. These investigations demonstrated that the more severe the initial septic event, the more morbidity and mortality were found in these particular patients. We propose that the immunosuppression associated with sepsis is, in part, a result of the prolonged activities of immunomodulatory chemokines such as CCL2 [monocyte chemoattractant protein-1 (MCP-1)], CCL17 [thymus and activation-regulated chemokine (TARC)], and C10 (CCL6), all of which are induced by interleukin (IL)-13 and IL-4. Thus, the pulmonary immune response in septic patients may be inappropriately skewed toward the T helper cell type 2 (Th2) cytokine pattern. This hypothesis is in keeping with published data showing that chemokine receptors have been found to be differentially associated with Th1/Th2 subsets [13], and CC chemokines appear to alter the outcome of the immune responses through altering the balance of Th1 and Th2 immune activation [14]. We contend that the balance of proinflammatory and modulatory CXC and CC chemokines is critical following a septic challenge, as this balance drives the response toward an efficient response (i.e., infection resolution) or toward an exaggerated release of immunomodulatory chemokines (i.e., inappropriate immune response and death). Herein, we provide data and discussion regarding the manner in which chemokines modulate the pulmonary innate-immune response after sepsis.

IMMUNOSUPPRESION FOLLOWING SEPSIS

A major research focus of several laboratories, including our own, is to elucidate the mechanisms that influence the developing, pathologic changes that occur during the initiation and maintenance of experimental sepsis. We have learned a considerable amount about the factors that initiate the early inflammatory events associated with sepsis, and these factors include a number of proinflammatory cytokines (including IL-1 and TNF). However, less is known about the long-term effects of sepsis on the immune system, particularly in the lung. This paucity led us to develop a murine model of severe sepsis induced by cecal ligation and puncture (CLP), a model that more closely mimics the clinical scenario. Following CLP surgery, C57Bl/6 mice were given 3 days of an antibiotic treatment regiment, which increased survival from 0 to 60%.

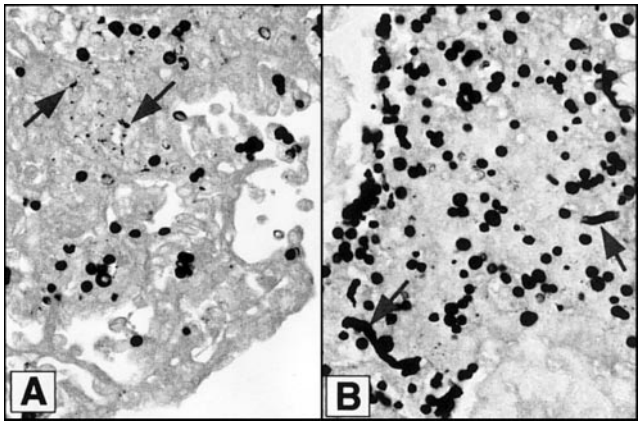

Fig. 1. Histological section of the lung at day 2 after an *A. fumigatus* conidia challenge in mice subjected to CLP 15 days previously. Sham (A) and CLP (B) mice were injected intratracheally with 5×10^7 *A. fumigatus* conidia, killed at day 2 after this fungal challenge, and the lung tissue were harvested and processed by routine, histological techniques to evaluate the presence of conidia and hyphae (black dots/arrows, ghost conidia in A and hyphae growth in B). Original magnification, 1000×. The slides were stained with Gomori-methanamine-silver, a specific stain for fungus, which appears black in the figure.

The survivors were subsequently challenged with *Aspergillus fumigatus* on the third day after CLP surgery. In this model, CLP groups, but not the sham surgery group, were clearly predisposed to the fungus challenge. The CLP survivors were also susceptible to a bacterial challenge with *Pseudomonas aeruginosa* (60% mortality) on the third day after CLP surgery. It is interesting that in a similar manner, all mice that survived the original septic episode died rapidly after a fungal challenge on day 15 after CLP surgery.

An assessment of the lungs of the mice surviving sepsis and receiving a subsequent fungal, pulmonary challenge demonstrated an interesting immune conundrum. In the CLP group that received *A. fumigatus* challenge, at day 3 or 15 post-surgery, a significant increase in the recruitment of leukocytes (i.e., macrophages and neutrophils) into the lung was apparent, as compared with the sham control animals. It is interesting that mice subjected to sham surgery effectively contained the fungus (**Fig. 1A**), whereas CLP mice were clearly not able to eliminate the fungal challenge, as evidenced by the presence of *A. fumigatus* conidia (or spores) and hyphae (Fig. 1B). Fungal growth to this degree is usually observed in severely immunocompromised patients or immunosuppressed animals [15–17]. These data suggest that a disconnect exists between the expression of inflammatory mediators needed to recruit leukocytes to the lung and the ability of the recruited leukocytes to destroy the fungal challenge.

This disconnect was confirmed by transmission electron microscopic analysis of lung samples at day 2 after *A. fumigatus* challenge in sham and CLP mice (**Fig. 2**). Dead or dying conidia (i.e., ghosts) were apparent in macrophages and neutrophils from the sham group (Fig. 2A), but in the CLP group, phagocytosed, intact conidia were observed (Fig. 2B). We also observed numerous hyphal elements in the lung of the CLP group but not in the sham group (Fig. 2C).

The reason for the impairment in the fungal killing in the CLP group is not presently apparent and is the subject of ongoing studies. Gene array analyses have revealed that a number of proinflammatory [examples include the p35 subunit of IL-12, IL-6, interferon (IFN), and IL-1β] genes were up-regulated in the sham group challenged with *A. fumigatus* compared with the sham group challenged with saline. However, no difference in the expression of these genes was observed in the CLP group challenged with the fungus compared with the sham group challenged with fungus. It is interesting that CCL2 transcript expression was greatly increased in CLP mice challenged with *A. fumigatus*. This finding has led us to investigate whether the sustained generation of CCL2 may lead to the suppression of macrophage activation. In support of this hypothesis are recent studies documenting that activated macrophages can be divided into three heterogeneous groups with distinct immunological functions. First, the "classic" or activated macrophage releases TNF, IL-1, IL-6, IL-12, and nitric oxide (NO) and has the ability to kill and degrade intracellular microorganism. Second, the "alternatively activated" macrophage releases IL-10 and IL-1ra, which does not produce NO, protects the host from an overzealous inflammatory response, and is involved in tissue repair. Third, the type II macrophage, which releases large amounts of IL-10 and induces T cells to produce IL-4, but this cell also produces IL-6 and TNF [18, 19]. The differential activation of macrophages may explain the "paralysis" of these cells in sepsis. Supporting this concept is

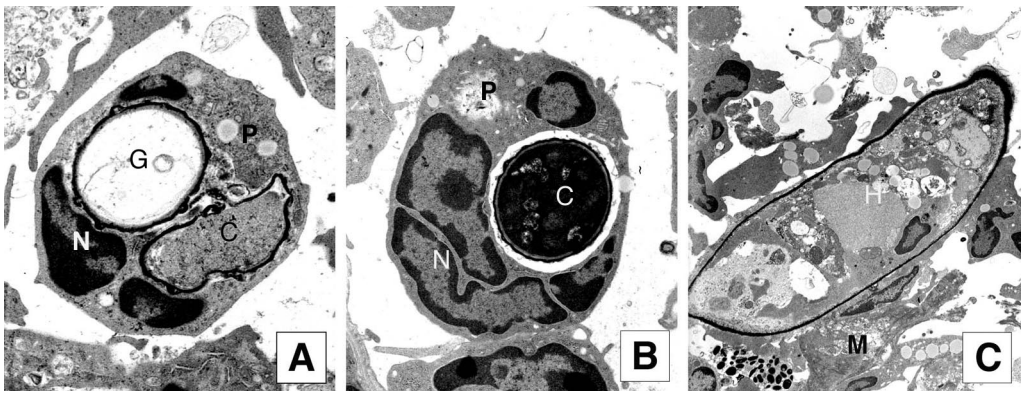

Fig. 2. Electron micrograph of the lung of sham (A) and CLP (B and C) groups challenged with *A. fumigatus*. Mice were injected intratracheally with 5×10^7 conidia of *A. fumigatus*, killed at day 2 after fungus challenge, and the lung tissue was harvested and processed for transmission electron microscopy. (A) Neutrophil (P) from sham-operated mouse with two conidia inside: a dead conidia—ghost (G)—and a degraded conidia (C). (B) Neutrophil from CLP-operated mouse with intact conidia inside (C). (C) Hyphae (H) in the lung of CLP mice with a macrophage (M) attached to the cell wall of the fungus. Original magnification, 2600×.

evidence that macrophages remain active after sepsis, but their repertoire of mediators is shifted away from those best suited for fighting pathogens [18, 19].

POTENTIAL STRATEGIES FOR OVERCOMING IMMUNOSUPPRESSION

Chemokine modulation of TLRs in lung

Recent evidence shows that TLRs recognize specific patterns of microbial components, and these receptors appear to regulate innate and adaptive immunity. There are several inherent levels of sophistication built into the TLRs, as different microbial structures are recognized by different TLRs. For example, TLR1 binds triacyl lipopeptides (bacteria, mycobacteria); TLR2 binds lipoprotein/lipopeptides, lipoteichoic acid (Gram-positive bacteria), and zymosan (fungus); TLR3 binds double-stranded RNA (virus); TLR4 binds LPS (Gram-negative bacteria); TLR5 binds flagellin (bacteria); TLR6 binds diacyl lipopeptides (mycoplasma); and TLR9 binds CpG DNA (bacteria). The ligands for TLR7, TLR8, and TLR10 are presently unknown. Activation of specific cells via TLRs can serve as potent signals for the expression of a number of mediators necessary to initiate and maintain an inflammatory response. In particular, the activation of the TLR2 or TLR4 pathway increases the expression of CXC (IL-8) and CC chemokines (regulated on activation, normal T expressed and secreted and MCP-1) [20, 21]. It is interesting that the expression of TLRs is not limited to leukocytes, as fibroblast and epithelial cells have been shown to have functional TLR2 and TLR4, and when activated, these receptors are involved in the expression of significant levels of chemokines [20, 22, 23]. The activation of TLRs leading to chemokine production is likely an important step required to rapidly engage the innate-immune response, thereby allowing the participation of specific leukocytes in the inflammatory response. However, more recent studies in our laboratory suggest that chemokines may account for the dysregulation of the TLR expression on macrophages from CLP mice. The dynamic interaction between chemokines and TLRs is likely an important cascade that may ultimately control the host's response to a foreign agent. It is elucidating that the manner in which chemokines regulate TLR expression may provide important clues to effective therapies in sepsis.

Chemokine modulation of DC recruitment to lung

Determining the overall contribution of DCs to the immunosuppression after sepsis is an area of ongoing investigation. Studies directed at the long-term fate of lung DCs are now appearing in the literature. For example, Wysocka et al. [24] showed that mice primed with LPS and then challenged with a lower LPS dose contained a significant reduced number or $CD11c^{high}$ IL-12-producing cells. As IL-12 is a critical cytokine needed to drive a Th1 response, the loss of DC cells from the lung may be an important factor in the impaired antipathogen or innate response in the CLP mice challenged with *A. fumigatus*. In addition, Barton and Medzhitov [25] have demonstrated a relationship among TLR activation, DCs, and the manner in which TLR–DCs control the adaptive immune response. DC subsets differ from each other via the expression of distinct sets of pattern-recognition receptors (such as TLRs) and by the cytokine/chemokines they produce upon maturation. In our immunosuppression model, we are presently examining whether modification of chemokine levels alters the presence and/or level of activity of DCs in the lung. Once again, this type of study may be directly relevant to clinical sepsis, where it has been shown that DC levels are reduced [25]. In addition to DCs, monocytes have been identified to play an interesting role in septic patients, as deactivation of this cell population has been reported to be associated with immunosuppression [26]. In particular, monocyte human leukocyte antigen (HLA)-DR expression is down-regulated during sepsis and is associated with a worse clinical outcome. The suppression of HLA-DR can be reversed by IFN-γ treatment, which in turn improves the clinical course of the disorder.

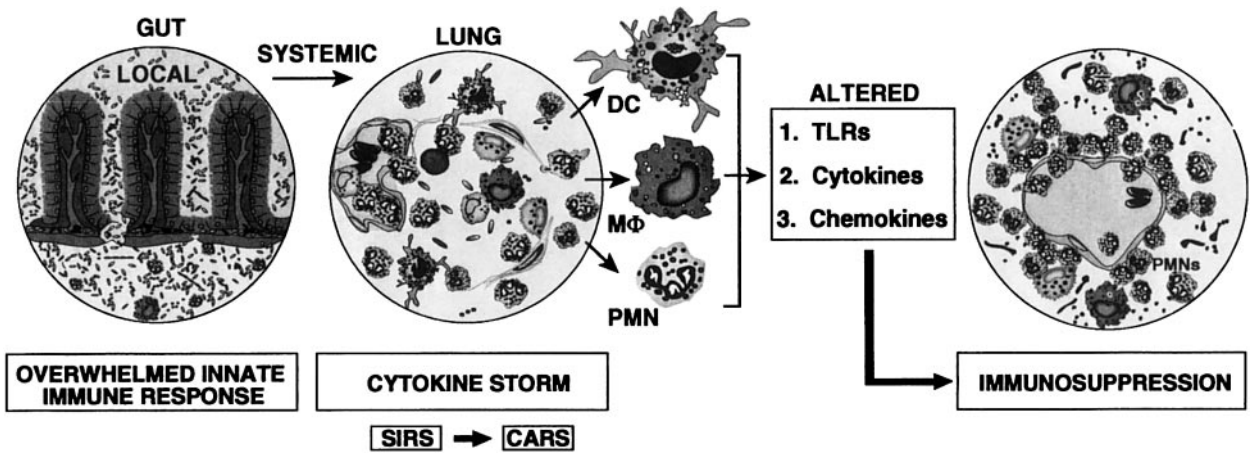

Fig. 3. A hypothetical sequence of events that leads to the pulmonary immunosuppression observed following sepsis. MØ, Macrophages; PMN, polymorphonuclear; SIRS, systemic inflammatory response syndrome; CARS, compensatory anti-inflammatory response syndrome.

CONCLUDING REMARKS

The preceding review suggests that severe diseases with a poor, long-term outcome may be a result of a dysregulated, inflammatory response. Sepsis is a severe acute disease that can result in immunosuppression, which leads to a secondary infection, thereby amplifying the risk of death. There is a critical need for mechanistic studies directed at understanding the immunosuppression induced by sepsis, mostly important for the elucidation of therapies that could enhance protective immunity against opportunistic pathogens. **Figure 3** outlines our evolving hypothesis in which a severe, overwhelming innate-immune response by the host (i.e., caused by rupture of the intestine) against enteric microorganisms affects organs including the lung. Within the lung, the release of inflammatory mediators such chemokines ultimately leads to a deviation in the inflammatory response. Although this response is clearly needed to shut down an exacerbated, inflammatory response as a result of sepsis, it is ultimately deleterious in the case of a secondary infectious insult. We also propose that cytokines (i.e., IL-13, IL-4, IL-10) and chemokines (i.e., C10, MCP-1, TARC) have major roles in the regulation of TLRs on macrophages and DCs during sepsis. The phenotype and activation state of macrophages may also be affected as described above. Understanding the cross-talk between soluble mediators and inflammatory cells may lead to immunotherapies that revert the immunosuppression and increase the survival rate following severe sepsis.

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