Inflammation and Sepsis: Past, Present, and the Future

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A critical burn injury is a unique trauma that often is accompanied by significant metabolic disturbances as well as perturbation of innate and adaptive immunity. Human skin is not only a barrier against environmental insults and against colonization of pathogenic microbes but, more importantly, it is an immune organ with significant surveillance and thermoregulatory functions. Therefore, it is hardly surprising that loss of large portions of skin as the result of burns results in impaired immunity, metabolic compromises, fluid shifts, and heat loss.

Through improvements in resuscitation, critical care, nutritional support, and early closure of the burn wound during the past two decades, our ability to care for burn patients has resulted in a marked reduction in morbidity and mortality. However, nosocomial wound or pulmonary infections, especially in patients requiring ventilator assistance, remain a major problem.

INFLAMMATION IN BURNS AND ITS CONSEQUENCES

Inflammation is an essential and primordial component of normal healing when wounds are small and localized. Prompt wound closure resolves this response. However, inflammatory responses in a critical burn injury often are deranged because there is global involvement of multiple tissue beds and their constituent immune and nonimmune cells, placing significant metabolic and energy requirements on the repair process. For example, the extent of inflammation and energy requirements is directly proportional to the severity of injury sustained by the patient. The spectrum of inflammation runs from a mild elevation in cytokines associated with inflammation that largely go unnoticed clinically to a systemwide severe inflammatory response that eventually leads to microcirculatory failure of capillaries supplying individual vital organs, acute respiratory syndrome, severe coagulopathy, and the development of the multiple organ failure.

Any critical injury of a large magnitude causes activation of multiple biological cascades in a temporal fashion. During this dynamic time course response, an acute phase of hyper-reactive immune response is followed by a hyporeactive phase. Traditionally, the hyper-reactive phase is often called “systemic inflammatory response syndrome,” or SIRS, and the subsequent hypo-reactive phase as “counter anti-inflammatory response syndrome,” or CARS. SIRS often is associated with enhanced production of proinflammatory cytokines and chemokines, increased free radical production through reactive oxygen species, and activation of complement and coagulation cascades. The net pathophysiological impact of these responses is manifested in increased vascular permeability, fever, leukocytosis, tachypnea, peripheral vascular resistance, and increased leukocyte mobilization and recruitment and the resultant bacterial killing. Activation of coagulation can precipitate disseminate intravascular coagulation, which increases the risk of mortality. Later during their clinical course, critically injured burn patients experience CARS, which is punctuated by the production of anti-inflammatory cytokines, leukocytopenia, severely impaired neutrophil phagocytosis, edema, and an increased risk for nosocomial infections.

INNATE AND ADAPTIVE IMMUNITY ARE THE EFFECTORS OF HOST-DEFENSE

Central to the host-defense in any severe injury is the robust and balanced participation of leukocytes, especially the polymorphonuclear neutrophils (PMNs)
and monocyte/macrophages.\textsuperscript{13} In a critical burn, host defense mechanisms become critical to the survival of the host. Significant loss of vital skin tissue signals inflammatory responses and recruitment of PMN and monocytes initially and T cells and B cells at a later stage.\textsuperscript{14} The influx of phagocytic cells is essential for controlling bacterial colonization and preventing the development of invasive wound infections but also serves the important function of clearing cellular debris and sets in motion the wound healing process.\textsuperscript{13} However, in large burns, especially in a full-thickness or a third-degree burn injury where there is a profound loss of keratinocyte progenitors that prevent wound closure through the growth of new skin, wound healing will not occur without successful skin grafting. Partial-thickness or second-degree burns, on the other hand, still contain much of the dermis and the keratinocyte progenitors and therefore will heal unless the wound site becomes infected. In the event of an infection, bacterial and fungal products damage or destroy the underlying tissue as the result of proteolysis and exotoxins. The consequence of such an infection can be a marked delay in wound closure or a conversion in the depth of injury equivalent to that of a full-thickness burn. Therefore, robust and continuous innate immune response to the burn wound site is of paramount importance to the wound closure, healing, and the clinical outcome of these patients.

**INNATE ADAPTIVE IMMUNE RESPONSE TO BURN INJURY**

The innate immune response to inflammation and sepsis is a rapid response that is not pathogen specific.\textsuperscript{15} It constitutes the first line of host-defense in humans and is a conserved response in all vertebrates. Leukocytes that mediate innate immunity are primarily PMNs, monocyte/macrophages, natural killer cells, and natural killer T cells.\textsuperscript{9,16} Although the biology of each type of leukocyte is often discussed individually in the context of any disease process, including severe trauma and burn injury, they seldom act alone. They communicate and interact with each other in the tissue microenvironment through the ligands they produce and respond to the matrix molecules that surround them. It is essential to understand that the concerted action of all the constituents of innate immune cell system is required if the functional integrity of the innate immune response is to be maintained.

Aside from limiting microbial entry and growth in the burn wound and underlying tissues, innate immunity forms the bridge to the longer-lasting pathogen-specific adaptive immune response. The immune cells that bridge the two arms of immune response are dendritic cells and macrophages, both of which can present pathogen specific antigens to T and B cells to initiate cellular and humoral immunity.\textsuperscript{17,18} Antigen presentation requires phagocytosis and intracellular killing of the pathogen, digestion of associated proteins and lipids, and presentation of the pathogen specific peptides through the MHC class II complex to the T and B cells.\textsuperscript{19}

In a critical burn injury in which inflammation is sustained for a significant period of time after the injury, demands placed on the innate and adaptive immune response are significant. The durability of the immune response under these conditions is dictated by the hormonal response to stress (catecholamines, glucocorticoids etc), the nutritional status, and the age of the patient as well any preexisting co-morbidities. In a burn patient with a significant injury alterations in the tissue microenvironment can further perturb burn induced metabolic demands and physiologic response and further increase the risk of contracting a nosocomial infection. The therapeutic interventions that are required in the care of a burn patient such as multiple surgical procedures, anesthesia, and blood transfusions add further to the risk for an infection.\textsuperscript{9,20}

Bacterial infections and sepsis, in particular, pose a significant clinical problem in all intensive care unit (ICU) patients, including those that are treated in the burn ICU. After the discovery of inflammation-associated cytokines, many immunotherapies directed at neutralizing these bioactive compounds have been tried. Although many of them yielded somewhat promising results in animal models, they have failed to produce meaningful treatment alternatives in large clinical trials.\textsuperscript{21,22} A potential reason for the ineffectiveness of many of the sepsis immunotherapies is perhaps attributable to the fact that they are monotherapies directed against a single component of the inflammatory cascade.\textsuperscript{23} The immune response to inflammation and infection is a complex cascade of pro- and anti-inflammatory molecules that change with the duration and severity of a critical injury. It is not surprising that an approach such as anti-tumor necrosis factor-\(\alpha\) antibody or IL-1 receptor antagonists have not proven to be effective treatment modalities in sepsis because the timing and intensity of the response to be manipulated in a given patient is dynamic and likely needs to modulated over time.

**MICROBIAL RESISTANCE TO ANTIBIOTICS AND BURN CARE**

One of the difficulties in effectively treating septic patients is the emergence of increasing number of
bacterial pathogens with resistance to multiple antibiotics. In recent years, Staphylococcus aureus, Pseudomonas aeruginosa, and Acinetobacter baumannii have posed a significant risk to critically injured burn patients because of their drug resistant patterns. In the United States alone, approximately 90,000 patients die of infections acquired during their stay in the hospital. According to Infectious Society of America, 70% of these mortalities are caused by multidrug-resistant bacterial strains.24 As the incidence of sepsis is rising, much of the current research in the fields of critical care and sepsis has focused on host-response.25 Although this focus has generated much useful information, unfortunately it has provided only a partial picture of the pathophysiology of sepsis.26 In the last 5 years through a nationwide multicenter effort spearheaded through a National Institutes of Health Inflammation and Host Response to Injury Glue Grant, we are beginning to accumulate extensive genomic and proteomic data from different tissues, including blood, skin, muscle, and fat from burn patients. Although the data collection phase are extensive, they are an essential component in our understanding of the importance of the genomic and proteomic oscillations in the pathophysiology of inflammation associated with burn injury, and sepsis.27–29 To date, no direct correlations have emerged from these studies. If such correlations are established, it will be necessary to test the hypothesis that they are indeed the causative factors and not associative responses to assure us of their utility in the development of either novel diagnostic or therapeutic modalities.

These potential advancements in understanding the human response to burn injury, however, do not diminish the ever-increasing problem of antibiotic resistance strains. One area of research in which significant improvements have not been made is in the development of new classes of antibiotics to overcome the threat. The number of newer antibiotics is currently 60% less than existed in the mid-1980s. Since 1960, only two new classes of antibiotics have been introduced for clinical use, linezolid in 2000 and daptomycin in 2003.24 In the light of these facts, how do we find new ways to meet these healthcare challenges?

**PATHOGEN–HOST RESPONSE**

Similar to the responses of the host to the pathogen centered on eradicating the microbial insult, the bacterial pathogen also uses specific mechanisms that allow it to evade immune detection and ensure its survival.30 Through processes such as adherence to host tissue, active evasion of the immune system cells, and direct damage to the host through exotoxin production, bacteria are able to initiate, disseminate, and sustain infections.31 To combat bacterial infections, especially multidrug-resistant nosocomial infections, and to devise pathogen specific antimicrobial therapy, it is essential to understand the interrelationship between the responses of the host and the bacterial pathogen.

Much our understanding of the host–response to Gram-negative bacterial infections has come from studies with purified lipopolysaccharide (LPS) in cell culture and whole animal studies. Unfortunately, in many nosocomial Gram-negative pathogens, LPS is a less pathogenic determinant compared with the multitude of exotoxins secreted by these pathogens that aid in their evasion and paralysis of our immunity and cause tissue damage. For example, 86 different exotoxins are secreted by *Pseudomonas aeruginosa*, which range from proteases including elastases, collagenses, peptidases to hemolysins and exotoxins A, T, U, and S, all of which can cause immune cell inactivation and tissue damage.32 Similarly, another difficult to treat multidrug-resistant pathogen, *Staphylococcus aureus*, secretes multiple exotoxins that are harmful to host tissues and facilitate in the dissemination and survival of the *Staphylococcus*.

In the last decade, it has become apparent that bacteria communicate with each other specifically through “quorum-sensing” mechanisms.33 Recent studies have begun to elucidate how bacteria proactively respond to our immune response while attempting establish an infection by turning on specific genes that counteract the killing ability of the phagocytes. For example, *Yersinia pestis*, the causative agent of bubonic plague, specifically up-regulates nitrosative stress response genes that neutralize intracellular nitric oxide-mediated bacterial killing during the early stages of infections, when it is most susceptible to elimination by PMNs and macrophages. However, when the *Yersinia pestis* migrates to the local lymph node, the achievement of quorum enables them to turn on Type III exotoxins, which are directly injected into immune cells through a needle like mechanism to inactivate the cytoskeleton, thus disabling their phagocytic capacity.34 Interestingly, the *Yersinia pestis* does not turn on oxyR or soxR genes that combat superoxide- and hydrogen peroxide-mediated killing as they are less susceptible to these agents.35 *Pseudomonas aeruginosa* on the other hand, up-regulates the transactivator OxyR to produce catalases and hydroperoxidases to neutralize hydrogen peroxide and hydroperoxide mediated killing by PMN and macrophages.36 Our own studies with oxyR deletion mutant in a *Pseudomonas aeruginosa* burn wound sepsis
model showed that OxyR expression is essential for inhibiting bone marrow progenitor differentiation into dendritic cells but not monocyte/macrophages, although these two cell types are derived from the same myeloid progenitor cell.\textsuperscript{37}

Therefore, the proper study of sepsis cannot just be a study of the host response to infection but must include understanding the inter-relationship between biological systems of the host and the pathogen that are engaged in a biochemical tango of survival. Such studies can be broadly classified as pathogen–host response. One may ask, what new information we might gain from this approach? Establishing the specific gene and protein expression patterns of bacteria at different stages of infection and sepsis and correlating them with corresponding host–response genomic and proteomic expression patterns opens the way to early detection of bacterial infections through better and high throughput pathogen specific diagnostics. This is essential knowledge if we are to tailor our antibacterial therapies to individual patient needs rather than on an empiric basis as is currently practiced. The clinical decision-making quandary that faces all critical care physicians is knowing when to start and when to stop treating a critically ill patient with antibacterial agents because of the lack of proper tools that will promote the practice of evidence-based medicine in this area.

Second, understanding the genomic and proteomic expression changes in bacteria during an infection or sepsis that are occur as an adaptive response in an attempt at evading immune detection and elimination will lead to a new class of antibacterials that are directed at preventing or impeding 1) the changes bacteria invoke to evade detection and 2) the toxins they produce to cause tissue destruction. For example, if we could devise compounds that delay or inhibit the establishment of “quorum” by inactivating quorum-sensing molecules such acyl homoserine lactones, we might have an opportunity to prevent the pathogen from becoming virulent by turning off their exotoxins.\textsuperscript{38,39} By preventing the adjustments that bacteria make during the process of establishing and disseminating an infection, we may be able to more effectively use currently available less toxic antibiotics perhaps at a lower dose for a shorter time. The most irresistibly logical reason to promote studies of pathogen–host response is that it provides the simultaneous opportunity to develop both better diagnostics and better primary or adjunct therapy for multidrug-resistant nosocomial infections that are currently a bane in our ability to care for critically injured burn patients.

**Table 1. Sepsis: the challenges and the unanswered questions**

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<tr>
<th>Question</th>
<th>Answer</th>
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<td>What are the critical control elements responsible for the transition from the “normal state” to the burn induced inflammatory response?</td>
<td>How does the dynamic time course of the inflammatory response evolve in a burn patient and impact their recovery and predisposition to infections?</td>
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<td>Could we effectively monitor and modulate innate and adaptive immune responses?</td>
<td>How does critical injury compromise immunity through imbalances in hormonal and metabolic demands?</td>
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<td>Given that multi-antibiotic-resistant bacterial strains are increasing at a rapid rate and that discovery of new classes of antibacterials is lagging, what are the potential options for the treatment of infections in critically injured burn patients?</td>
<td>Will understanding host–pathogen responses open pathways to new and rapid diagnostics and potential tailored antibacterial therapies based on interruption of metabolic actions of bacteria to host initiated defense mechanisms?</td>
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**PRIORITY IN SEPSIS/INFLAMMATION: BREAK-OUT SESSION DISCUSSIONS**

The major unanswered questions regarding sepsis and inflammation in burns, as listed in Table 1, were used to begin the dialog for the round table discussions. Several specific areas for future research were defined. (Table 2) These areas can be divided into four categories: definitions, early identification, treatment optimization, and mechanistic studies.

Although sepsis, SIRS, and infection have been defined for a variety of populations, the pathophysiology of the burn wound and the host’s response to the burn make the application of these definitions problematic. The key question discussed was: Do the current definitions meet the needs of burns? Current definitions are frequently used as proxies for quality or standard of care; thus, the accuracy of these definitions assumes increased importance. The group concluded that a consensus needs to be reached on the definitions of infection, sepsis, and SIRS to provide a framework for research and for objective evaluation of patient outcomes.

Infection increases hospital costs, decreases patient survival, and has long-term effects on patient outcomes. Early detection of infection yields improved
survival; therefore, the second priority for inflammation research is to develop methods for early detection of infection. Potential areas of investigation include identifying markers (serum, genomic, physiologic) for early identification of infection/inflammation, development of a system for stratification of inflammation severity, and identifying methods of preventing/containing infection once it occurs.

Although the early identification or avoidance of infection will decrease the incidence of sepsis, the treatment of infection and sepsis continues to be a priority. Currently, the treatment of infection in burns centers on the use of antibiotics. Optimal use of antibiotics, including antibiotic type, timing of antibiotic administration, and length of antibiotic use, need to be defined. The role of antibiotic rotation to avoid the emergence of resistant pathogens in the burn center may decrease the number and severity of nosocomial infections. Finally, the role of gut decontamination needs to be defined in burns. This priority for burn research will involve both clinical and basic science studies: we need to understand the mechanisms behind the efficacy of antibiotics and the inflammatory process to design appropriate therapeutic choices.

The final priority discussed in the breakout session was the determination of the role of inflammation in burns. Although inflammation can often be deleterious after burn injury, it is also necessary for wound healing and patient recovery. One of the key research questions relating to burns is: When does inflammation become deleterious and how do we identify this time point? The answer to this question will need to factor for multiple issues, including burn size, patient age, genomics, and environmental factors. Multiple agents have been utilized to modify the inflammatory response, including glycemic control, beta blockade, and the use of anabolic agents. The timing and use of these agents needs to be defined through further prospective, randomized trials and mechanistic studies.

Table 2. Research priorities for sepsis

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<th>Priority</th>
<th>Description</th>
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<td>Define infection, sepsis, and systemic inflammatory response</td>
<td>syndrome in the burn patient</td>
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<tr>
<td>Develop methodology for early identification of infection</td>
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<tr>
<td>Optimize the treatment of infection in burns, including the use of</td>
<td>antibiotics, gut decontamination, and length of treatment</td>
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<tr>
<td>Determine the role of inflammation in burns and how we can</td>
<td>modify the inflammatory response to improve outcomes</td>
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CONCLUSIONS

Inflammation and sepsis represent major challenges to the critically ill burn patient, and studies improving our knowledge of inflammation and sepsis after burn injury are vital to improving patient outcomes. The top research priorities for infection and sepsis involve defining infection and sepsis, identifying patients at risk, optimizing treatment, and modifying the inflammatory response. Accomplishing these goals will require the integration of clinical and basic science techniques and knowledge, as well as support for multidisciplinary projects.

REFERENCES