

Commentary

Heat shock protein gene expression and survival in critical illness

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Introduction

The advent of recombinant DNA technologies has created unprecedented opportunities for significantly improving the treatment and prevention of human disease. Recombinant DNA technology has made it possible to study the molecular factors that modulate cellular responses to various metabolic and environmental stresses. Incorporating molecular biology techniques into the research arsenal of the physician will provide the opportunity to dissect and define the reversible and irreversible intracellular processes that give rise to acute respiratory distress syndrome, sepsis, septic shock, and multiple system organ failure (ie the major causes of mortality in most respiratory and multidisciplinary intensive care units). Primarily, this short review discusses a specific example of gene expression that has been studied over the past 20 years, and its relevance to acute lung injury and sepsis, two of the most common causes of critical illness.

Acute lung injury is defined as a phenomenon of acute diffuse lung inflammation. When inflammatory cells are sequestered and activated in the lung, they release potentially toxic metabolites, proteolytic enzymes, and an array of cytokines. Survival of patients who have acute respiratory failure after acute lung injury depends on prompt alveolar repair. Even when the original inciting factor is controlled, outcome varies from complete recovery to death. The survival rate approximates 50% in all major series [1] and the major determinant of outcome is the failure of other vital organ systems. Approximately 90% of all deaths occur within 2–3 weeks of the onset of the syndrome.

Gene expression in the lung during critical illness

The cellular events involved in lung inflammation, and organ damage and repair are ultimately controlled at the molecular level and cannot be fully understood without consideration of the functions of the relevant genes and their gene products. It is now widely recognized that various cellular stimuli mediate their physiologic effects by the induction of complex intracellular signaling cascades, which culminate in the activation or induction of a particular gene or subset of genes. By extension, activation leads to the synthesis of particular sets of proteins and a consequent change in cellular behavior. Depending on the nature of the pathologic perturbation, these steps represent potential targets for interventional maneuvers and novel therapeutic strategies.

In considering the pathogenesis of organ inflammation and damage, neutrophils, monocytes, macrophages, and platelets represent cell populations that are likely to be etiologically relevant and that therefore represent logical cell populations to assess in relation to altered gene expression during organ failure. Similarly, epithelial and endothelial cells respond to injury with acute alterations in mediator generation and surface molecule expression [2,3], and appear to act in concert with inflammatory cells to influence the tissue response to injury and inflammatory stimuli. These interactions in turn are known to induce the expression of various genes that encode proteins that are central to coagulation, fibrinolysis, and repair. Among these proteins, a critical set of molecules that are involved

in directing the inflammatory response is the family of cytokine proteins.

Cytokines, a word derived from the Greek terms for 'cell' and 'mover', are low-molecular-weight glycoproteins that may act locally in a paracrine or autocrine manner to influence cell behavior or may act more generally to induce substantial systemic effects. The locally active cytokines are often produced by T-lymphocytes, whereas cytokines produced by macrophages are often active systemically. Advances in DNA technology have had a major impact on the identification of specific cytokines and the definition of their roles in tissue injury. Molecular studies of cytokine expression have revealed that the induction of marked increases in the expression of certain cytokines after tissue injury often correlates with the magnitude of tissue damage [4]. Among the cytokines that have been particularly well studied in relation to tissue damage [2–5] are tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-2, IL-6, IL-8, interferon- γ , transforming growth factor- β , and platelet-derived growth factor. The influence of these and other cytokines on a particular cell or cell population may be greatly influenced by interactions with other cytokines and other types of regulatory factors. For example, the combined effects of IL-1, TNF- α , and lipopolysaccharide are believed to be responsible for the activation of epithelial and endothelial cells during endotoxemia [6]. These cytokine–cellular interactions produce acute changes in cell functioning in response to local injury, and may be associated with normal growth and repair when functioning appropriately, or alternatively with disease pathogenesis if cytokine production becomes uncontrolled. Thus, inappropriate cytokine production and/or cell response to cytokine stimuli may lead to ongoing inflammation and chronic disease.

Sepsis represents one of the most challenging problems in the field of intensive care medicine. Even with the use of powerful antimicrobial agents, sepsis continues to represent the most common cause of respiratory failure, multiple system organ dysfunction, and death in patients admitted into intensive care units. Cumulative experimental and clinical evidence indicate a major role for cytokine production and systemic release in sepsis-induced inflammatory responses. Thus, blocking cytokine activation and/or pharmacologic effects with specific cytokine-receptor antagonists represents a logical strategy for the treatment or attenuation of sepsis-related inflammation [2–7]. Although strategies that facilitate selective downregulation of the effects of specific cytokines would be particularly attractive from the therapeutic perspective, achieving this objective is far from straightforward, because the effects of specific cytokine inhibition *in vivo* have proved to be extremely unpredictable. For example, despite data from animal studies that show a dramatic efficacy of antibodies against endotoxin and TNF, and IL-1 receptor antagonist

in the treatment of sepsis [7], corresponding beneficial effects have not been observed in recent human trials [8–10]. Accordingly, it appears that information concerning cytokine biology will need to be considerably improved before contemplating the development of a 'magic bullet' that will attenuate inflammatory responses during organ injury and prevent organ dysfunction.

Heat shock response and heat shock protein gene expression

From bacteria to humans

The eukaryotic cell response to many potentially deleterious exposures is remarkably similar to that of prokaryotic cells. This similarity is particularly well exemplified by the so-called heat-shock response [11], a response to stress that involves the rapid induction of a set of highly conserved genes that encode heat shock proteins (HSPs). The heat shock response has been observed in virtually all organisms, including plants and bacteria, as well as invertebrates and vertebrates. Mammalian cells have been shown to synthesize HSPs after a brief period of hyperthermia (temperatures 3–5°C above normal body temperature), but the genes that encode HSPs can also be induced by a variety of other stimuli, including environmental modifications, such as prolonged ischemia, sodium arsenite, ethanol, salicylates, and viral infections, and agents that affect cell cycle [11]. HSPs may be induced directly by such agents, or indirectly by virtue of increased expression of other proteins that in turn provoke HSP gene expression. Thus, for example, increases in HSP expression in injured cardiac muscle cells have been linked to increases in TNF- α and IL-1 production [12].

HSPs are classified into five protein families on the basis of molecular mass. These include the large molecular weight HSPs (100 kDa); the HSP-90 family; the highly conserved HSP-70 family, which represents the most prominent eukaryotic group of HSPs; the HSP-60 family, members of which are found in bacteria, chloroplasts, and mitochondria; and the small HSP family, members of which are expressed predominantly in plants. Comparison of the sequences of the respective heat shock genes from bacteria, plants, flies, and humans have indicated these genes to be among the most highly conserved proteins in nature. In addition to this structural conservation, these genes are also remarkable in their capacity to be rapidly induced in response to a broad spectrum of stimuli. At least 10 HSP-70 related genes have been found in the human cells, some of which map to between the complement and TNF- α and TNF- β genes on chromosome 6 [13].

The HSPs appear to manifest many diverse functions. It has been suggested, for example, that members of the HSP-70 family act in the protection of cellular damage by binding to denatured or abnormal proteins after heat shock, thereby preventing protein aggregation [14].

Perhaps the most compelling argument that HSPs have protective functions is the phenomenon of thermotolerance [11–14]. Thermotolerance represents a property of all living cells and refers to the capacity of cells to survive or recover from normally lethal exposures to abrupt, severe heat shock or stress conditions if, before the lethal stress, the cells are exposed to milder or shorter periods of heat/stress conditions. Such 'tolerizing' to cellular stress has been shown to reduce the extent of heat-induced central nervous system injury markedly [15]. Although the mechanism for HSP-mediated cytoprotection is not understood, one possible explanation is that this protective effect relates to the capacity of HSPs to block the synthesis of cytokines such as IL-1 β that play key roles in the febrile and inflammatory responses to stress [16,17].

Preventing organ injury

Irrespective of the mechanisms by which the stress response provides cytoprotection, the capacity of HSPs to subserve this function is of considerable interest from the perspective of elucidating the pathophysiology of organ damage and dysfunction. Accordingly, a number of recent studies have addressed the question regarding whether the induction of the stress response might protect animals against subsequent injury [18–22]. Our group [19–22] has examined the effects of the induction of the heat shock response by whole-body hyperthermia in attenuating lung damage and outcome in different experimental models of direct and indirect lung injury. Those studies demonstrated that a brief exposure of experimental animals to transient hyperthermia, resulting in HSP-72 protein accumulation in the rat lung, attenuated lung damage and significantly decreased mortality.

To investigate whether the protective effect of the heat shock response could be generalized to other models of acute lung injury and could prevent or reduce extrapulmonary organ injury and death, we chose to study this effect in a rat model of intra-abdominal sepsis produced by cecal ligation and perforation [21]. This experimental model mimics many features of the human septic syndrome, with the presence of enteric micro-organisms and endotoxin in the blood. We studied two groups of animals (heated and unheated), and evaluated survival rates and pathologic changes in the lung, heart, and liver before and after cecal perforation, after cecum removal, and at 7 days. At 18 h after perforation, 25% of the unheated animals died, whereas none of the heated animals died. Seven days after cecal perforation, the protection was still evident, with 20 and 70% mortalities in the nonstressed and heat-stressed groups, respectively. In addition, heated animals showed less histologic evidence of lung and liver damage.

Because whole-body warming could be associated with a number of nonspecific mechanisms that are unrelated to the induction of the heat shock response, Ribeiro *et al* [22]

used sodium arsenite as a nonthermal means to induce the heat shock response and examined whether this could also provide protection in the same model of intra-abdominal sepsis. Following a single intravenous injection of sodium arsenite, HSP-72 was detected in the lungs, with a peak between 18 and 24 h after the insult. Administration of 6 mg/kg sodium arsenite 18 h before performing cecal ligation and perforation was associated with a marked decrease in mortality at 18 and 24 h after sepsis. The protection in the sodium arsenite-treated animals appeared to follow the time course of HSP-72 protein levels.

All of these studies support the hypothesis that HSPs are cytoprotective *in vivo*. The mechanisms by which the heat shock response might provide cytoprotection are not known. Ribeiro *et al* [23] have demonstrated recently, in endotoxin-stimulated alveolar macrophages, that HSP-72 coprecipitated with TNF- α from cells that had received stress treatment (heat stress and sodium arsenite) before endotoxin exposure. This finding suggests that HSPs may participate in post-translational control of TNF- α release, making HSPs responsible for decreased TNF- α release by binding TNF- α intracellularly and preventing its release from macrophages. Therefore, HSPs determine whether TNF- α is released from the cell or is sent to the lysosomal machinery for degradation.

Deshpande *et al* [24] reported in the present issue of *Critical Care* that the induction of heat shock response, before sepsis, markedly decreased lactate concentration in plasma in septic rats. Lactic acidosis develops as a result of organ hypoperfusion. Several published clinical studies during the 1970s and 1980s showed a correlation between high levels of lactate and poor outcome in patients with sepsis and septic shock. By contrast, low levels of lactate and/or an ability to increase lactate clearance has been associated with good prognosis. Although the mechanisms by which the induction of the heat shock response might improve organ perfusion and attenuate organ damage are not fully understood, it has been demonstrated that the heat shock response inhibits cytokine-mediated expression of inducible nitric oxide synthase [25]. As Deshpande *et al* have pointed out [24], organ protection may depend of the degree and duration of the heat stress. Although a mild stress induces a protective response, a more potent stress stimulus induces apoptosis, and an even stronger one leads to necrosis.

Conclusion

A further understanding of the role of the heat shock response might allow for the development of rational pharmacologic agents and to make them potential targets for therapeutic interventions. Future research could focus on novel strategies to activate the HSP genes, as a potential therapy for sepsis, acute lung injury, and other critical care conditions.

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