Basic science review

Recent Insights into the Pathogenesis of Severe Sepsis

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ABSTRACT

Objective: *Severe sepsis remains the dominant challenge in the care of critically ill patients. Over the last 10 years a large body of research has modified our understanding of this condition. In this article, we review the evolution of our understanding of the molecular mechanisms responsible for the development of this clinical syndrome.*

Data sources: *The authors undertook a critical review of the literature on the molecular basis of the pathogenesis of sepsis with particular emphasis on the role of cytokines, toll-like receptors, adhesion molecules, coagulation cascade molecules and the possible role of in-vitro experimental models of blood-endothelium interaction.*

Summary of review: *Recent insights into the molecular mechanisms responsible for the pathogenesis of the severe sepsis syndrome suggest that pro- and anti- inflammatory pathways are simultaneously activated and interact in a dynamic way. Pro-inflammatory cytokines previously considered as targets for intervention have typically been already activated and de-activated by the time the clinical diagnosis is made and intervention is possible. Cellular activity involving white cell-endothelial interactions occur later, making them a more attractive option for therapeutic intervention. Immunological incompetence rather than over-activity may be the most common state of cell function in critically ill patients.*

Conclusions: *Our understanding of the the pathogenesis of severe sepsis continues to grow. Expression of membrane surface molecules such as toll-like receptors, adhesion molecules and cytokine receptors induce a high degree of redundancy and amplification. Cell responsiveness is reduced in an attempt to circumvent the amplification loop. However, the ensuing interaction between the host and the pathogen(s) may lead to an immune deficiency, leaving the field open to further invasion by the original bacteria or to superimposed infection agents. Endothelium-white cell interactions might be an appropriate target for future interventions.* (**Critical Care and Resuscitation 2005; 7: 32-39**)

Key words: Sepsis, systemic inflammatory response syndrome, multiple organ failure syndrome

Severe sepsis and septic shock are the primary causes of the multiple organ dysfunction syndrome (MODS). Many water-soluble mediators with pro- and anti-inflammatory actions such as tumor necrosis factor-α (TNF-α) and interleukins (IL-6, IL-8, IL-10) appear to play a core role in sepsis.

Acute renal failure (ARF) is increasingly seen as part of MODS in critically ill patients.[1,2] MODS is the most frequent cause of death in patients admitted to ICU.[3] Severe sepsis and septic shock are the primary

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causes of MODS[4,5] which develops largely as a result of the host response to Gram-negative and Gram-positive bacterial infection.[6]

Local infection develops into a systemic inflammatory response syndrome (SIRS) that encompasses a complex mosaic of interconnected events including the so called compensatory anti-inflammatory response syndrome (CARS). Several studies have examined the mechanisms responsible for the diffusion from a local site to systemic involvement. In principle, four classes of microorganisms (i.e. bacteria, fungi, viruses and parasites) are responsible for infection. Past and recent epidemiologic data show that trends in microbial resistance in Europe are overlapping with those observed in the United States of America. Bacteria (90%) and fungi (10%) cause the majority of infections in developed countries. An increase in age-adjusted death rates due to bacteraemia, from 4.2 per 100,000 population in 1980 to 13.2 per 100,000 in 1992 was detected in the United States of America.[7] Antibiotic consumption is nearly ten times greater in the ICU than in general medical wards. The widespread use of broad-spectrum antibiotics has fuelled the emergence of resistant micro-organisms. The development of newer and more potent antimicrobial agents such as linezolid and quinupristin/dalfopristin against Gram positive bacteria can not keep up with the increasing anti-microbial resistance. Within the "European prevalence of infection in intensive care" (EPIC) study, both Gram-positive and Gram-negative infection were common with at least 60% of each group of organisms being resistant to commonly used antibiotics.[8] Antimicrobial resistant bacteria are increasingly widespread in European hospitals[9] and independently associated with higher morbidity and mortality, longer hospitalisation and increased hospital costs compared with antibiotic-susceptible bacteria (e.g. median hospital charges were $52,971 for patients with methicillin-sensitive S. aureus infection and $92,363 for patients with methicillin-resistant Staphylococcus aureus [MRSA] infection in hospitals).[10]

A recent hypothesis suggested that a defective host innate response renders bacteria resistant to host recognition and defence mechanisms, leading to systemic infection and sepsis.[11] In higher organisms, a variety of host defence mechanisms control the resident microflora and, in most cases, effectively prevent invasive microbial disease. Many microbial pathogens avoid host recognition or dampen the subsequent immune activation through sophisticated interactions with host responses. Some pathogens even benefit from the stimulation of inflammatory reactions. A defective response of the host may depend on a unique genetic makeup of a pathogen that can render it more resistant to antibiotics or by disturbances in the integrated response of both the innate and adaptive arms of the immune system. Differences in reactivity of dendritic cells to microbial molecules through toll-like receptors (TLRs) are associated with susceptibility and resistance to microbes.[12] Molecules such as bacterial lipopolysaccha-rides (LPS), microbial lipopeptides, microbial DNA, peptidoglycan and lipoteichoic acid trigger the interact-ion with the toll-like receptors and related molecules (MD-2, MyD-88), the principal sensors of the innate immune response.[13-15]

Stimulus-receptor coupling activates different signal transduction pathways leading to exacerbated generation of cytokines, and phospholipase A_2-dependent, arachid-onic acid-derived platelet-activating factor, leukotrienes and thromboxanes. Within the plasma, activation of the complement (C3a, C5a, and their products) and coag-ulation factors, interact with the process as products generated in the fluid phase may, in turn, trigger and sustain cell activation. Other agents play a role in the pathophysiology of sepsis such as surface-expressed and soluble adhesion molecules, kinins, thrombin, myocard-ial depressant substance(s), endorphin and heat shock proteins.

The pathogenesis of sepsis

In physiological conditions, the biological activity of sepsis-associated mediators is under the control of specific inhibitors that may act at different levels. In sepsis, the homeostatic balance is altered and a profound disturbance of relative production of different mediators may be observed.[16] On the one hand, the 'spill' into the circulation of mediators intended to have autocrine or paracrine effects generates systemic effects including endothelial damage,[17] procoagulant, fibrinolytic, comp-lement activities, haemodynamic shock and vaso-paralysis.[18-24] On the one hand, monocytes are unable to produce cytokines when they are challenged with different stimuli, *ex vivo*.[25,26]

The pathogenesis of sepsis was initially described as an overproduction of host pro-inflammatory factors. The concept was established on the basis of several studies. The injection of LPS into experimental animals and healthy human subjects reproduces the initial phase of bacterial infection.[27] In humans, LPS alters capillary integrity and affects the cardiovascular system,[27] causes production of cytokines,[27-29] and activates the coagulation-fibrinolytic pathways.[30] Peak concentrations of IL-1, TNF-α, IL-6 and IL-8 occur within 2 - 3 hours of LPS infusion.[19,20] Studies in mice have shown that ICAM-1 mutant mice are resistant to the lethal outcome of endotoxin-induced pneumonitis.[31]

What is the relevance of circulating cytokines? The presence or absence of detectable levels of cytokines

within biological fluids reflects a rather complex balance between enhancing and inhibitory signals acting on producer cells, between production and catabolism, and between their binding to the target cells and the modulation of their receptors on the cell surface.[16] Furthermore, their presence does not necessarily parallel their activity. A possible interplay between a given cytokine and its relative inhibitor should also be considered.[16] Cavaillon *et* al,[16] coined the expression of circulating cytokines as being "the tip of the iceberg" implying that neither their presence nor their absence can reflect the complex interplay at the tissue level. Despite the fact that their peak concentrations may reflect an exacerbated production, these levels do not necessarily stand for enhanced bioactivity. The concept of sepsis as a simply pro-inflammatory event has been subsequently challenged.[25,26,32,33]

In sepsis and SIRS, cell-associated cytokines in peripheral blood monocytes are decreased as is the capacity of these cells to produce several cytokines such as TNF-α, IL-1α, IL-1β, IL-6, IL-10 and IL-12,[34-36] (but not IL-1 receptor antagonist).[37] Hyporesponsiveness is not only present in monocytes but also occurs in whole blood[38] and is associated with increased plasma levels of IL-10 and prostaglandin E_2 which are potent inhibitors of the production of pro-inflammatory cytokines.[35,36,39] To describe the excessive anti-inflammatory counterpart of SIRS, Bone *et al*,[40] coined the acronym CARS for "compensatory anti-inflammatory response syndrome". Terms such as monocyte deactivation, immuno-paralysis or, more simply, cell hyporesponsiveness all indicate the inability of cells to respond *ex vivo* to LPS stimuli due to overproduction of anti-inflammatory cytokines. Adib-Conquy *et al*[41] demonstrated that, upon LPS activation, peripheral blood mononuclear cells of patients with SIRS show patterns of NF-κB expression that resemble those reported during LPS tolerance: global down-regulation of NF-κB in survivors of sepsis and trauma patients and the presence of large amounts of the inactive homodimer in non-survivors of sepsis.

In the clinical setting of severe sepsis, Bone *et al*,[40] proposed that, at a given time, SIRS or CARS may predominate in patients, inducing shock or immune depression. However, a large amount of evidence now suggests that in patients with both infectious and non-infectious sepsis, SIRS and CARS may co-exist but in different compartments (Figure 1).

Based on these concepts, Cavaillon *et al*,[33] recently proposed that SIRS predominates within the inflamed tissues, while blood leukocytes show hyporeactivity. A restrained inflammatory response within the blood-stream should avoid the endothelial activation leading to over-expression of adhesion molecules, adherence and degranulation of leukocytes. It would avoid fatal clotting and organ failure. Nevertheless, peak concentrations of either LPS or different cytokines have been variably reported, and in some reports their levels, alone or in combination, have even been considered as useful markers in severity scores.[42]

Following the intravenous injection of LPS, a lag phase occurs and is followed after 1 hr by a steep increase in TNF reaching a maximum at 1.5 hr. TNF plasma concentrations are sharply reduced at 3 hr when both IL-6 and IL-8 sharply increase. Cytokine production is blunted by cyclooxygenase inhibition.

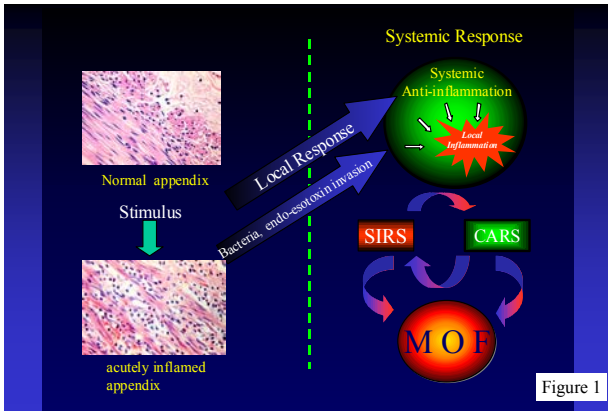

Figure 1. The complex mechanisms of the innate response are usually able to localise an inflammatory process thus preventing it to diffuse systemically. However, in conditions that favor such diffusion (genetic predisposition, altered immune response), the initial, pro-inflammatory response may be uncontrolled and cause the systemic inflammatory response syndrome (SIRS). A compensatory anti-inflammatory response syndrome (CARS) may also be triggered. Multiple organ failure (MOF) is characterised by the alternate presence of SIRS and CARS or by their co-existence but in different tissues.

Studies by Suffredini *et al*,[27] Parrillo *et al*,[43] and van Deventer *et al*,[20] on human subjects infused with LPS characterised the initial inflammatory (TNF-α, IL-6, IL-8) and haemostatic (TAT, PAP, tissue plaminogen activator) responses out to 6 - 8 hours. Taylor *et al*,[23] studied the receptor and oxidative enzymatic responses of phagocytes in the human model of endotoxemia and correlated them with the response of molecular markers of haemostatic and inflammatory system activation and endothelial injury. These authors established that the compensated response to LPS consists of two stages: an immediate symptomatic inflammatory stage followed by an asymptomatic stage that is characterised by a recurrence of haemostatic activity, appearance of complement activation products complexed to C-reactive proteins and evidence of endothelial injury.[23] In particular, they showed the occurrence of a two-step response: an early response characterised by the degranulation of neutrophils (as detected by elevation of

elastase-α1anti-trypsin complexes) coinciding with peak concentrations of TNF-α, IL-6 and regulatory responses such as IL-10 and activated protein C; a late response characterised by haemostatic activity as reflected by the appearance of a second a large peak of soluble fibrin
and a second and greater decrease in circulating factor VIIa concentration coupled with the appearance of increased concentrations of plasma tissue factor antigen. This second phase was also characterised by sustained, markedly elevated levels of C-reactive protein (CRP), and CRP-bound activated complement components.[23] A crucial aspect of these studies is that peak concentrations of LPS and of several cytokines appear at different time intervals. The intravenous injection of pro-inflammatory cytokines (TNF-α, IL-1) reproduce very closely the pattern of haemodynamic and intravascular changes induced by the injection of LPS.[23] Recent evidence also suggests that activated protein C provides protection from LPS-mediated microcirculatory dysfunction by systemic anti-inflammatory effects on leukocytes and endothelium subsequently improving capillary perfusion.[44]

In the human setting of sepsis, the finding of elevated levels of different pro- and anti- inflammatory cytokines has been variable, suggesting the possibility that their production may occur at a different time from sampling, or that their production in a given patient is compartmentalised. The events associated with sepsis/ SIRS may happen in sequence (the sequential or serial sepsis theory) whereby pro- and anti- inflammatory mediators are alternatively produced in high or low generation periods, thus ensuing in SIRS and CARS. On the other hand, the events associated with sepsis/SIRS may occur simultaneously (the parallel sepsis theory) in that SIRS and CARS may coexist in different areas or systems. In the sequential sepsis theory, temporary prevalence of SIRS should probably be treated with high dose steroids assuming that a timely intervention is possible due to early and accurate biological monitoring. Otherwise, the therapy may overlap with the next period of CARS and may then favor bacterial colonisation and infection dissemination. Indeed, in this period, protect-tive antimicrobial therapy or even immuno-stimulatory therapy should be administered. The time of intervention becomes crucial in order to prescribe the right therapy for the right disorder. Alternatively, if the parallel sepsis theory is considered, neither therapy may result in an adequate response and accordingly a 'question mark' exists on the most sound therapeutic option.

In intensive care practice, blocking any one mediator has not led to measurable outcome improvement in patients with sepsis.[45] Possibly more rigidly defined subgroups would benefit by anti-TNF treatments.[46] On the other hand it has been shown that antagonising a cytokine may lead to deleterious consequences and substantially higher mortality.[47] A low-level TNF-α response seems to be necessary for the host defense to infection[48,49] as well as high levels seemingly needing to be modulated by an anti-inflammatory feedback. In sepsis, however, impaired regulation may cause an excessive anti-inflammatory response which generates monocyte "immunoparalysis" and exposes the host to further infections. Both processes (inflammation and anti-inflammation) are designed to act in response to specific stimuli in a well-balanced fashion defined as immuno-homeostasis. Furthermore, the time point of therapeutic intervention in the septic process seems to be crucial. As the network acts like a cascade, early intervention would seem most beneficial. On the other hand, sepsis does not fit a one-hit-model but shows complex and multiple rises in mediator levels that change over time. Therefore, neither single-mediator-directed nor one-time interventions seem appropriate. One of the major criticisms attributed to continuous blood purification treatments in sepsis - its lack of specificity - could turn out to be a major strength. Non-specific removal of soluble mediators - be they pro- or anti-inflammatory - without completely eliminating their effect may be the most logical and adequate approach to a complex and long-running process like sepsis.

Endothelium and endothelium-dependent mechanisms of leukocyte recruitment in sespis

Endothelium is recognised as an endocrine/paracrine structure which secretes several vasoactive mediators or autacoids that decisively affect vascular tone and platelet function. The endothelium lining the circulatory system serves as an important target and a modulator for the effects of endotoxin because of its close contact with circulating blood and its proximity to the underlying vascular smooth muscle. Prolonged periods of sepsis and septic shock result in the development of endothelial dysfunction.[50] It is likely that an evolving stromal environment plays a key role in the development of chronic inflammatory pathology, in part at least by modifying the responses of endothelial cells (ECs). Recruited leukocytes including T-cells and monocytes may also provide positive feedback.[51] Monocytes are able to directly induces ECs to upregulated adhesion receptors and support capture of flowing leukocytes. This pro-inflammatory capability may decline during normal maturation into macrophages.[52] However, in abnormal tissues the monocyte-derived cells may themselves take on

phenotypes that promote a pro-inflammatory state *via* interaction with endothelium.[53]

Endothelium-derived substances (e.g. nitric oxide and endothelin)[54] are regarded as key mediators in SIRS that lead to fatal multiple organ dysfunction. In several experimental models, endotoxin has been shown to increase the constitutive release of nitric oxide by the endothelium and the activity of inducible nitric oxide synthases (iNOS), that may contribute to the deleterious effects of endotoxin.[55] The role of endothelin in sepsis is similar; in particular an excessive rise in the plasma level of endothelin for a long period evokes profound vasoconstriction. Increasing blood flow to the mesenteric circulation by endothelin blockade, i.e. using a non-specific endothelin receptor antagonist (e.g. bosentan), seems to be a promising strategy in the therapy of septic shock.[56]

Leukocyte recruitment by endothelium during inflammation

Endothelial cells contribute to the regulation of vascular tone, haemostasis, angiogenesis and passage of soluble compounds and cells of the immune system to, and from, tissues. Of particular importance during inflammatory responses is the ability of vascular endothelial cells to both initiate and control the recruitment of leukocytes.[57] They achieve this by responding to cytokines and other inflammatory agonists, to modify surface expression of adhesion receptors and chemokines required for the capture of leukocytes from the blood stream and migration into tissue. Endothelium in venules of lymphatic organs is specialised for continuous capture and migration of recirculating lymphocytes, and post-capillary venules of other tissues are typically the dominant site for phased recruitment of granulocytes and mononuclear cells following an inflammatory insult.

The process of leukocyte recruitment can be divided into four steps; initial contact (tethering), rolling, firm adhesion and migration, with each step being mediated by adhesion molecules expressed on the surface of activated endothelium (Figure 2).

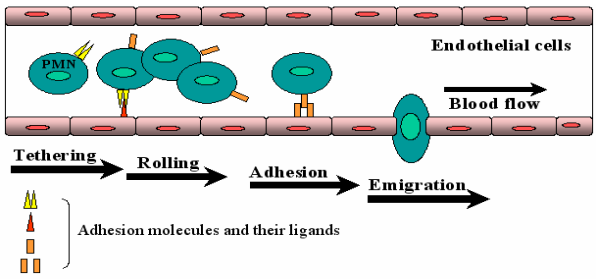

Figure 2. A schematic representation of the complex mechanisms involved in the tethering, rolling, activation, adhesion and emigration of leukocytes.

Leukocyte recruitment is designated as a cascade because interruption of the initial tethering and rolling prevents subsequent leukocyte adhesion and emigration. Initial capture is know to be mediated by endothelial adhesion molecules, including P-selectin, E-selectin and vascular cell adhesion molecule (VCAM-1). P-selectin is rapidly expressed on the endothelial surface in response to histamine, thrombin, and reactive oxygen metabolites. Associated with the rapid expression of P-selectin is the early recruitment of neutrophils on this selectin. By contrast, E-selectin expression requires de novo synthesis in response to inflammatory cytokines such as IL-1, TNF-α and LPS, with maximal amounts occurring about 4 to 8 hours after stimulation. The expression of E-selectin coincides with further recruitment of neutrophils and monocytes populations. However, in vitro experiments show that this selectin can support rolling of eosinophils and lymphocytes.[58] Numerous investigators have demonstrated that thrombin, a serine protease, may affect leukocyte infiltration into inflamed tissue, impacting at each stage of the cascade of events that lead to leukocyte recruitment. Thrombin has been shown to induce rapid P-selectin mobilisation to the surface of endothelium to induce leukocytes rolling.[59] In addition Kaplanski *et al*,[60] and Woodman *et al*,[61] have documented a dramatic increase in E-selectin-dependent leukocyte rolling 4 hr post thrombin exposure of endothelium.

The second phase of leukocyte recruitment, firm adhesion, can also be induced by thrombin as a result of rapid endothelial platelet-activating factor production and IL-8 production. These chemo-attractants found on the endothelium surface then activate rolling neutrophils so they can adhere to constitutive and newly-synthesised intercellular adhesion molecule-1. Additionally, throm-bin can activate the production of TNF-α and IL-1β to further amplify leukocyte recruitment.[62] In conditions like ischaemia-reperfusion of the mesenteric bed, where thrombin is a dominant pro-inflammatory molecule, anti-thrombin attenuates selectin-dependent leukocyte rolling and subsequent adhesion and vascular dysfunction. There are some studies to suggest that anti-thrombin III can reduce sepsis-induced neutrophils recruitment into the lung.[63]

A model to study leukocyte/endothelium interaction in sepsis under flow conditions

Emigration of leukocytes from blood into tissue requires a complex array of molecular and cellular events between the leukocyte and the endothelium. Development of the parallel plate flow chamber that simulates physiologic flow has been an important tool for clarifying the molecular events occurring between leukocytes and endothelium (Figure 3).[64] This flow

chamber consists of: 1) a base plate with an entrance and exit port through which cells and media are perfused, 2) a glass or plastic slide plate on which the substrate or cellular monolayer is placed, 3) a gasket that controls the chamber diameter, and 4) a vacuum outlet so that the apparatus can be held in place.[65]

This novel approach to study leukocyte-endothelium and leukocyte-immobilised protein interactions has many advantages. First, the shear forces better reflect shear forces observed in vivo and second, the system incorporates red blood cells that clearly impact on leukocytes tethering to substratum.

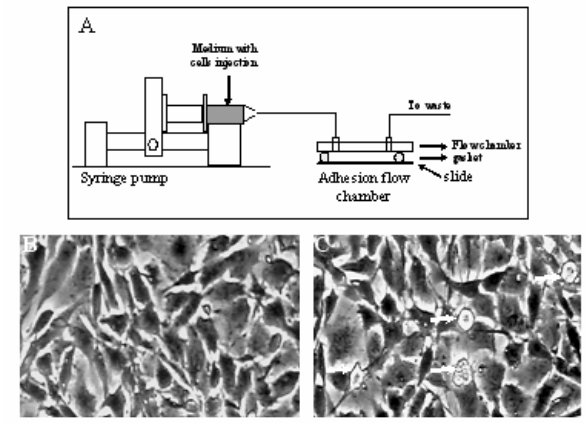

Figure 3. A schematic representation of the adhesion assay; B: K1 cells (renal clear cells carcinomas) were perfused on a monolayer of Eck33 cells (endothelial cancer cells); C: cells were considered firmly adherent after 10 sec of stable contact with the monolayers.

Although it is well appreciated that initial flow chamber work of single concentrated population of cells was critical to the understanding of the function of adhesion molecules, this new approach extends those studies to establish which adhesion molecules and combination of adhesion molecules will recruit selective populations of leukocytes. Moreover, the perfusion of whole blood over endothelium stimulated with various cytokines (i.e. TNF-α and IL-4) will provide further insight into which cytokines recruit which type of leukocytes. This technology can also be used for patient blood to establish whether the pathology of a particular disease may be an inherent humoral problem either of enhanced adhesion of leukocytes or enhanced activity of plasma. Indeed, Kubes *et al*,[66] have demonstrated a very dramatic increase in leukocyte-substratum interactions in septic patients.

The pathogenesis of sepsis continues to present unresolved questions. A complex cross-communication between the different cells (e.g. monocytes/macrophages, polymorphonuclear neutrophils, endothelial cells, and tissue resident cells) is mediated by a vast array of mostly water-soluble mediators. Expression of membrane surface molecules such as toll-like receptors, adhesion molecules, cytokine receptors induce a high degree of redundancy and amplification. Cell responsiveness is reduced in an attempt to circumvent the amplification loop. However, the ensuing interaction between the host and the pathogen(s) may be compromised leaving the field open to further invasion by the original bacteria or to superimposed infection agents. In the context of MODS, functional rather than histologic changes are observed in many of the failing organs, such as the kidney. The final biological expression of mediators produced in excess may depend on their concentration gradient, the responsiveness of the target cells and the type of cells. Cell activation, may lead either to further recrutiment of inflammatory cells or to cell necrosis or apoptosis. The expression of biological effects may thus produce important consequences on the recovery processes which may effect functional recovery.

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