Immunopathogenesis of severe sepsis

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Severe sepsis refers to the syndrome of fever, hypotension and organ dysfunction resulting from infection, commonly due to Gram-negative or Gram-positive bacteria with or without bacteraemia. Occasionally, non-bacterial pathogens, including fungi, rickettsiae, protozoa and viruses, can cause a similar syndrome. The first line of defence against infecting organisms comprises highly conserved interactions of the host's innate immune system with microbial products, followed by amplification of the immune response to maximise host defences. Whilst evidently conferring an evolutionary survival advantage, the humoral and cellular responses which ensue are directly involved in the pathogenesis of severe sepsis.

Host-microbial interactions

Microbial products which activate the innate immune response include both cell wall components and secreted proteins (Table 1).

Cell wall components

Lipopolysaccharide (LPS), or bacterial endotoxin, forms a major portion of all Gram-negative cell walls and is the most important microbial product implicated in sepsis. It has a structurally conserved lipid component, lipid A, and an oligosaccharide portion composed of a core region and highly variable O-specific chains. Lipid A activates multiple components of the innate immune system. Cellular activation occurs predominantly by binding to a serum protein, LPS binding protein, which facilitates interaction of LPS with the cell surface receptor CD14. Transmembrane signalling through recently described 'Toll-like' receptors follows. Monocytes, macrophages and neutrophils express membrane CD14. LPS activation of cells such as endothelia, which do not express the membrane bound form, can take place via soluble CD14. Other cell surface receptors, such as the integrin CD11/18 or the macrophage scavenger receptor which can bind LPS, may also be involved. LPS activates both classical and alternative pathways of complement, leading to production of anaphylotoxins C3a and C5a and terminal C5b-9 complexes. Direct activation of Factor XII of the intrinsic clotting cascade or contact system stimulates coagulation, fibrinolytic and plasma kallikrein-kinin systems. Gram-positive bacterial cell wall components such as peptidoglycans and lipoteichoic acids similarly demonstrate CD14 dependent cellular activation. They can also activate complement and contact systems, although the mechanisms are poorly understood.

Secreted proteins

Secreted bacterial toxins which can cause sepsis fall into several groups. So-called 'superantigens' produced by Staphylococcus aureus and Streptococcus pyogenes can induce potent non-specific

| Table 1. Some examples of microbial products implicated in the pathogenesis of severe sepsis. |
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| **Cell wall components** | Lipopolysaccharide | All Gram-negative bacteria |
| | Peptidoglycan | All Gram-positive bacteria |
| | Lipoteichoic acid |  |
| **Secreted toxins** | Staphylococcal toxic shock syndrome toxin 1 | S. aureus |
| | Staphylococcal enterotoxin A | S. pyogenes |
| **Proteases** | Phospholipase C | Clostridia |
| | Lipase | S. aureus |
| | Nuclease |  |
| | Neuraminidase | S. pneumoniae |
| | Streptococcal pyrogenic exotoxin B | S. pyogenes |
| **Porins** | α-Haemolysin | E. coli |
| | Leukocidin | S. aureus |
| | Catalase |  |
| | Pneumolysin | S. pneumoniae |
| | Streptolysin S & O | S. pyogenes |
activation of monocytes, macrophages and T cells by cross-linking major histocompatibility complex class II molecules with subsets of T cell receptor Vβ chains, without intracellular processing² (Fig 1). Alternatively, bacteria can provoke a host response by causing cellular injury by production of pore forming toxins and a variety of proteolytic enzymes. Some bacterial protease enzymes stimulate the immune system directly by cleavage of inactive substrates to active pro-inflammatory mediators, such as release of bradykinin from high molecular weight kininogen or the conversion of the pro-form of the cytokine interleukin (IL)-1 to its active form by streptococcal pyrogenic exotoxin B². Although there may be subtle differences in the way in which Gram-negative and Gram-positive bacteria interact with the host⁶, many similarities are evident in the events that follow activation of the innate immune system, irrespective of the infecting organism.

Amplification of the host response

Following the initial host-microbial interaction, widespread activation of other components of the innate immune response takes place, schematically outlined in Fig 2. This pro-inflammatory cascade is mediated by either direct cell-cell interactions or soluble factors derived from serum proteins and cells, which act in autocrine, paracrine or endocrine fashion.

Fig 1. Schematic outline of lipopolysaccharide (LPS) and superantigen (in contrast to conventional antigen) mediated cellular activation (APC = antigen presenting cell; LBP = LPS binding protein; MHC = major histocompatibility complex; TCR = T cell receptor; TLR = Toll-like receptors).

Fig 2. Schematic outline of the interactions between cellular and humoral components which amplify the innate immune response to infection (IFN = interferon; IL = interleukin; PAF = platelet activating factor; TNF = tumour necrosis factor).
Cell-cell signalling

Direct cellular interactions occur through constitutive or inducible expression of cell adhesion molecules (CAMs). This occurs between monocytes or macrophages and T cells during superantigenic activation, and between monocytes or neutrophils and endothelia during cellular extravasation and infiltration into tissues. Several families of CAMs involved in these cell-cell signals are well characterised, in particular the interaction of selectins with glycoprotein ligands such as the sialylated Lewis X antigen and that of β2 integrins, such as CD11/18 with members of the immunoglobulin supergene family, intercellular CAM (I CAM)-1/2 and vascular CAM (V CAM)³.

Plasma proteins

Factor XII activation simultaneously induces the fibrinolytic and intrinsic clotting cascades. Widespread activation of these pathways during sepsis leads to disseminated intravascular coagulation (DIC)⁷. Bradykinin, also generated from contact system activation, causes vasodilatation and increased capillary permeability, as do C3a and C5a fragments from the complement cascade which are neutrophil chemotactants, thereby increasing inflammatory exudates⁹.

Cytokines

Activated monocytes, macrophages, neutrophils and endothelia produce small soluble proteins, grouped together as cytokines. These provide important amplification signals in the immune response to infection and have been directly implicated in the pathogenesis of sepsis¹⁰. In particular, tumour necrosis factor (TNF-α) and IL-1 are produced very soon after cell activation and are potent stimuli for activation of similar cells locally and distally. IL-6 is the major acute-phase stimulant inducing de novo hepatic acute phase protein synthesis. As part of the acute phase response, cytokine induced synthesis of complement and coagulation factors, for example, may contribute to the amplification of these plasma protein cascades. Amongst other important cytokines, Interleukin-8 (IL-8) is a potent neutrophil chemotactant; IL-12 from monocytes and interferon γ produced by T cells form a paracrine amplification loop; platelet activating factor (PAF) acts on endothelia, increasing vascular permeability, augmenting the clotting cascade and the production of an inflammatory exudate. PAF is also a neutrophil chemotactant and induces superoxide generation and degranulation¹¹. Many non-cytokine mediators derived from activated cells are also evident. Nitric oxide (NO), originally identified as endothelium-derived relaxing factor, is produced by the enzymatic action of nitric oxide synthase (NOS) on L-arginine. Different isoforms of NOS are classified by virtue of constitutive (cNOS) or inducible (iNOS) expression. cNOS is produced by endothelia and regulates vascular tone, whilst iNOS, produced primarily by monocytes and macrophages in response to microbial products and activation by cytokines, causes NO-mediated vasodilatation and increases endothelial permeability, thereby enhancing inflammatory exudates¹². NO also reacts with superoxide anions generated by respiratory bursts in phagocytic cells to form peroxynitrite which has direct antimicrobial activity. Other mediators released by activated cells include tissue thromboplastin, which activates the extrinsic clotting cascade, histamine and eicosanoids such as prostaglandins (PGs) which similarly enhance inflammatory exudates.

Numerous negative feedback mechanisms are employed by the host to regulate amplification of the innate immune response (Fig 3). Severe sepsis is thought to occur when the pro-inflammatory cascade escalates beyond the control of anti-inflammatory regulation.

Pathogenesis of clinical features in severe sepsis

Fever

Thermoregulation is achieved by balancing heat production and heat loss strategies under control of the autonomic nervous system to maintain a hypothalamic temperature set-point. In sepsis, the cytokines IL-1, IL-6 and TNF-α stimulate endothelia and astrocytic terminals in the perivascular spaces of the hypothalamic organum vasculosum laminae terminals to produce PGE₂.¹³ This, in turn, inhibits thermosensitive neurons and raises the hypothalamic temperature set-point. The autonomic nervous system then increases heat production and reduces heat loss until the new set-point has been reached. In severe sepsis, hypothermia can supervene for reasons that are not clear, but may reflect compensation of thermoregulatory mechanisms rather than a low hypothalamic temperature set-point. Heat loss increases with peripheral vasodilatation, and heat production decreases as organ failure ensues.
Hypotension

Many of the products of cellular and plasma protein activation, in particular NO, bradykinin, complement anaphytoxins, prostacyclin, histamine and PAF induce vasodilatation in the microvascular circulation by a direct effect on vascular smooth muscle cells. Inflammatory exudates also cause local tissue hypoxia and hypoperfusion. Homeostatic neurohormonal mechanisms attempt to compensate, producing a hyperdynamic circulation typical of sepsis, characterised by decreased systemic vascular resistance and increased cardiac output. In severe sepsis, cardiac output falls, in part due to hypovolaemia resulting from increased capacitance of dilated blood vessels and fluid extravasation due to increased vascular permeability. Cardiogenic shock is also evident, due to poorly characterised myocardial depressant factors, one component of which is NO-mediated inhibition of positive inotropic and chronotropic responses to β-adrenergic stimulation. Coronary hypoperfusion and inflammatory infiltration of the myocardium also contribute to the immunopathogenesis.

Organ dysfunction

The typical manifestations of organ dysfunction are listed in Table 2. Their pathogenesis in sepsis is complex, multifactorial and incompletely understood. Tissue hypoperfusion and hypoxia are dominant factors. The mechanisms for this include widespread fibrin deposition in DIC causing microvascular occlusion, inflammatory exudates, and vasoactive substances resulting in abnormalities of microvascular homeostasis and systemic hypotension. The metabolic acidosis that supervenes further disables normal biochemical processes. Cellular infiltrates, particularly neutrophils, damage tissues directly by releasing lysosomal enzymes and superoxide-derived free radicals which peroxidate cell membrane lipids. As tissue injury progresses and organ function is com-

| Tissue          | Clinico-pathological features                                      |
|-----------------|-------------------------------------------------------------------|
| Brain           | Cerebral infarction                                              |
|                 | Diffuse micro-abscesses                                          |
|                 | Encephalopathy                                                   |
| Heart           | Myocardial inflammatory exudate                                  |
|                 | Myocardial depression                                            |
|                 | Arrhythmias                                                      |
| Lung            | Diffuse alveolar inflammatory exudate                             |
|                 | Acute lung injury                                                |
|                 | Acute respiratory distress syndrome                              |
| Kidney          | Acute tubular necrosis                                           |
| Liver           | Hepatocellular necrosis                                          |
| Gastrointestinal tract | Ischaemic necrosis                                                   |
| Skin            | Purpura                                                          |
|                 | Ischaemic necrosis                                               |

Fig 3. Anti-Inflammatory mediators and their actions (IL = interleukin; LPS = lipopolysaccharide; MIF = macrophage migration inhibitory factor; TAF = transforming growth factor; TNF = tumour necrosis factor).

Table 2. Common clinico-pathological manifestations in severe sepsis.
promised, for example by exacerbation of hypoxia due to lung injury, sequential multiple organ failure follows. Organ dysfunction in sepsis is covered in more detail in the accompanying article by Singer.

**Conclusion**

Whilst an infecting organism may produce toxins which injure tissues directly, this is often inadequate to explain the clinico-pathological sequelae in severe sepsis. Instead, the dominant role in pathogenesis may lie with components of the host immune response to infection. The highly conserved responses of the innate immune system comprise sequential activation and amplification of humoral and cellular antimicrobial defence mechanisms which can escape the control of anti-inflammatory regulation, inadvertently causing injury to the host. Further understanding of the immunopathogenesis of severe sepsis may unveil new opportunities for therapeutic intervention.

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**Pathophysiology and management of meningococcal septicaemia**

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**Neisseria meningitidis** (meningococcus) is a major infection risk globally. In the UK, it is the leading cause of death from infection in childhood, with a mortality around 10%. Most deaths from meningococcal infection are due to the development of fulminant septic shock. Yet *N. meningitidis* is a frequent commensal of the human upper respiratory tract. Carriage rates increase from less than 1% in infancy to a maximum of 25% in adolescence, declining to around 10% in adulthood.

The meningococcus is a Gram-negative diplococcus. Pathogenic meningococci possess a polysaccharide capsule, differences in the structure of which form the basis of separation into subgroups. The lack of suitable vaccines for all the meningococcal serogroups is because of a high level of genetic diversity caused by intraspecies recombination and transformation. A single mutation or genetic exchange may lead to an outbreak of clinical disease if associated with a change in an immunologically important surface antigen.

**Epidemiology**

Meningococcal disease is endemic worldwide. Serogroups B and C predominate in the UK with an incidence of 5–6 per 100,000. In sub-Saharan Africa, serogroup A predominates in cyclical epidemics every eight years and can affect up to 1,000 per 100,000 of the population. The reasons for regional variation in disease-causing serogroups are not well defined.

**Immunopathology**

**Transmission**

Transmission is by close contact or respiratory droplet spread.

**Colonisation and invasion of nasopharyngeal epithelium**

The risk of colonisation may be enhanced by disruption of the respiratory epithelial cell layer by irritants (such as cigarette smoke) or by a preceding viral illness, for example influenza. Binding to epithelial cells is established by pilus and outer membrane proteins. Certain outer