Inflammatory cell activation in sepsis

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The body relies for protection on an effective inflammatory response. To sustain an armoury of inflammatory cells in a state of permanent activation would be impossible and a system whereby such cells can be rapidly activated is, therefore, employed. Upon transition from the resting to activated state inflammatory cells perform multiple defensive functions and are then removed, limiting the duration of inflammation. Neutrophils are the major circulating inflammatory cells but macrophages exert a more powerful regulatory effect. If the inflammatory response is inadequate there is a risk of overwhelming sepsis. By contrast, an unregulated response can lead to systemic inflammation and consequent multiple organ damage. This review focuses on the mechanisms whereby inflammatory cells are activated, how the regulatory system may misfunction and how it may in the future be manipulated to therapeutic advantage.

Inflammatory cells are a vital part of the armoury against infection but, when inappropriately or excessively activated, can induce a state of uncontrolled systemic inflammation. This chapter focuses on the mechanisms whereby inflammatory cells are activated and upon the clinical problems associated with failure of the normal homeostatic mechanisms.

Serious infections, such as pneumonia and septicaemia, are the most common cause for admission to intensive care. Such infections and other non-infective (e.g. multiple trauma and burns) conditions commonly progress in the critically ill to a state of generalised inflammatory activation. This is characterised by the presence of a severe clinical illness and the presence of two or more of the following: a temperature of greater than 38°C or less than 36°C, a heart rate greater than 90 beats/min, tachypnoea of greater than 20 breaths/min or an arterial blood gas with a partial pressure of carbon dioxide (PaCO₂) of less than 32 mmHg, or a white blood cell count of greater than 12 x 10⁹/l or less than 4 x 10⁹/l or more than 10% band forms on the peripheral blood film. This multi-system inflammatory state is now known as the systemic inflammatory response syndrome (SIRS) and it is characterised by excessive immuno-inflammatory cascade activation leading to a widespread reduction in cellular oxygen utilisation, ATP depletion, cell injury and death. As a consequence of this generalised
activation, SIRS is commonly associated with multi-organ dysfunction syndrome (MODS), typified by the acute respiratory distress syndrome (ARDS)\textsuperscript{2}.

Inflammation requires the activation of numerous component systems including the leukocytes, the endothelium and multiple mediator networks that are normally quiescent, leading to the classic clinical condition recognised as \textit{calor, rubor, tumor and dolor}\textsuperscript{3,4}. The inflammatory cells are the main driving force behind this process and comprise the circulating leukocytes (neutrophils, monocytes and lymphocytes), in addition to tissue fixed macrophages, dendritic cells, mast cells and eosinophils. Neutrophils and monocytes constitute the bulk of circulating inflammatory cells. They normally exist in a non-activated state and in the absence of stimulation have a life-span limited by apoptosis (or programmed cell death) to a day or so\textsuperscript{5}. These cells are rapidly transformed by invading bacteria, specific bacterial products, foreign material, endogenous mediators or in response to trauma or hypoxia into highly active phagocytes with a greatly enhanced capacity to release mediators, enzymes and reactive oxygen intermediates (ROI)\textsuperscript{6}.

Activation increases the number of neutrophils by speeding the maturation process, increasing the release of precursors from bone marrow. Likewise the number of monocytes entering the circulation from the bone marrow is doubled during inflammation\textsuperscript{7}. Chemotactic agents and adhesion molecules focus these cells upon sites of infection where they phagocytose and kill bacteria. Monocytes mature into macrophages, which having engulfed, killed and digested micro-organisms, present their foreign antigen to lymphocytes and engender highly specific adaptive immune responses. These inflammatory cells also release a wide range of mediators which act to regulate the whole inflammatory process, controlling cellular activation, endothelial and leukocyte adhesion molecule expression and function, acting as chemotaxins, prolonging the life-span of inflammatory cells, stimulating fibroblasts and promoting wound healing and angiogenesis\textsuperscript{8,9}. Thus activation of the inflammatory cascade depends upon appropriate activation of the endothelium, the immune system, the cytokine/chemokine and other soluble mediator systems along with activation of inflammatory cells. However, inflammatory cell activation is the central trigger driving this whole process (Fig. 1).

**Hyper and hypo-responsiveness**

The ability to activate and de-activate the inflammatory process is central to homeostasis. A state of persistent inflammatory cell activation would be unsustainable and life threatening, by adversely influencing the normal flow of leukocytes through capillary networks resulting in
INSULT

- Local release of priming/activating agents
- Activation of inflammatory cells
- Release of:
  - Cytokines, Free radicals, Lipid mediators
    (appropriate balance of pro- and anti-inflammatory mediators)
- Up-regulation of adhesion molecules
- Generation of a chemotactic gradient
- Rapid inflammatory cell influx to site of injury
- Enhanced phagocytosis and bacterial killing
- Clearance of initiating insult
- Abolition of chemotactic gradient and activating factor production
- No further inflammatory cell influx
- Neutrophil apoptosis and phagocytosis by macrophages
- Macrophage emigration

RESOLUTION OF INFLAMMATION

widespread leukocyte plugging, globally increasing metabolic demand and leading directly to tissue damage through the release of free radical species. Clinical conditions occurring as a consequence of excessive activation of the inflammatory processes are now well described and include ARDS, disseminated intravascular coagulation (DIC), ischaemia reperfusion injury and MODS. By contrast, an inability to activate inflammatory cells would leave the body defenceless, as exemplified by neutropenia. Consequently, many endogenous mediators act to regulate the pro-inflammatory cascade. This down-regulating response has led to the term compensatory anti-inflammatory
Fig. 2 Hypo and hyper-responsive inflammatory states.

An inflammatory challenge may lead to an effective response with clearance of the initiating insult but the body may also respond inappropriately as shown in this figure. Compensatory anti-inflammatory response syndrome (CARS), systemic inflammatory response syndrome (SIRS) and multi-organ dysfunction syndrome (MODS).

As with over-activation, states of inappropriate hypo-responsiveness have been described. Critical illness can lead to impaired inflammatory cell responses with reduced neutrophil chemotaxis and bactericidal activity after burn injury or haemorrhage. Indeed, recent evidence suggests that persisting inflammation induces immune hypo-responsiveness with reduced monocytic capacity to present antigens and diminished superoxide generation. This is identified by reduced monocyte expression of HLA-DR, a state that may be reversed by treatment with interferon-γ (IFN-γ). It is, therefore, clear that inflammatory cell activation is not absolute; different cells being activated to different degrees via single or multiple different effector functions, all requiring a remarkable degree of co-ordination (Fig. 2).

**Priming and activation**

Cellular activation is classically described in terms of priming and activation. Priming is defined as a process whereby the response of an inflammatory cell to an activating stimulus is significantly potentiated by prior exposure to an appropriate priming agent. Normally, neutrophils express only limited microbicidal capacity when challenged. However, if first exposed to a priming agent, their response to a subsequent activating stimulus can be increased up to 20-fold. Priming agents alone (at priming concentrations) do not elicit such responses, although many priming agents may, if encountered at higher concentrations, also act as activating agents. The molecular species initiating the priming response does not have to be the same as that resulting in activation. Priming probably has...
Table 1 A list of known priming and activating agents

<table>
<thead>
<tr>
<th>Priming agents</th>
<th>Activating agents</th>
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<tbody>
<tr>
<td>ATP</td>
<td>IL-1β</td>
</tr>
<tr>
<td>PAF</td>
<td>PAF</td>
</tr>
<tr>
<td>TNFα</td>
<td>TNFα</td>
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<tr>
<td>IL-8</td>
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<tr>
<td>LPS</td>
<td>LPS</td>
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<tr>
<td>GM-CSF</td>
<td>PMA</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>C5a</td>
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<tr>
<td>Adhesion molecule cross-linking</td>
<td>fMLP</td>
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its most important clinical consequence in exaggerating clinical responses to further inflammatory triggers, with evidence for example of hypoxia increasing the subsequent pro-inflammatory effects of endotoxin\(^\text{15}\). Another simple clinical correlate would be the deleterious effect of a significant pre-existing infection on the outcome of elective major surgery. A list of some of the known priming and activating agents is shown in Table 1.

As there are many similarities between neutrophil and macrophage activation, both are initially considered together, specific differences will be highlighted later.

Source of activating agents

Exogenous activators

The best-described activating agent is lipopolysaccharide (LPS) or endotoxin. This component of the bacterial cell wall is common to all Gram-negative bacteria and can exert multiple effects if released systemically. LPS exerts many of its actions through the cell surface receptor CD14 that is expressed mainly on inflammatory cells. Binding to this molecule requires the presence of circulating factors such as lipopolysaccharide binding protein (LBP) or the group of plasma proteins known as the septins\(^\text{16}\). Other bacterial agents can exert similar effects, for example lipoteicoic acid, the peptidoglycans and N-formyl-L-methionyl-L-leucyl-phenylalanine (fMLP) from Gram-positive bacteria, all of which have distinct receptor populations.
Endogenous activators

The inflammatory cells are the main source of endogenous pro-inflammatory agents or cytokines, although the endothelium and mesothelium, amongst others are also able to elaborate these de novo. The classic pro-inflammatory cytokines are tumour necrosis factor α (TNFα), interleukin-1β (IL-1β) and interleukin-6 (IL-6)\(^\text{17}\). TNFα is detectable within 30 min of bacterial challenge, IL-1 within 3 h and IL-6 within 6 h. TNFα and IL-1 stimulate the release of other cytokines while IL-6 stimulates acute-phase protein production. There are multiple other mediators involved in the acute inflammatory response including the chemokines, the complement, kinin, coagulation and fibrinolytic cascades and lipid mediator synthesis, all occurring as part of an interrelated network (reviewed by Abraham\(^\text{18}\) and Foëx & Shelly\(^\text{19}\)).

Signal transduction

Priming and activating agents bind to their specific surface receptors, which initiate intracellular signals leading to one or more functional activation responses. These intracellular signal transduction pathways are now the subject of detailed investigation. LPS and other activating agents are now known to activate specific intracellular pathways including mitogen activated protein kinase (MAPK) and the transcription regulating factor nuclear factor κB (NFκB) that may provide novel therapeutic targets in the future (reviewed by Abraham\(^\text{18}\), Calkhoven & Ab\(^\text{20}\) and Schulze-Osthoff et al.\(^\text{21}\)).

Priming and activating agents act to regulate multiple cellular functions including: (i) adhesion molecule expression; (ii) phagocytosis; and (iii) cell killing. They also regulate the secretion of chemotaxins, enzymes and lipid mediators and can determine cell survival.

Adhesion molecules

As most infections, such as pneumonia or peritonitis, occur initially in the tissue and not in the blood stream, extravasation of circulating leukocytes is essential in bring inflammatory cells and pathogens into contact. Although circulating neutrophils and monocytes inevitably come into contact with the endothelium, binding to this layer is limited in the resting state. In the presence of pro-inflammatory cytokines or other activating stimuli, there is a co-ordinated up-regulation of endothelial and inflammatory cell adhesion molecule expression and
function\textsuperscript{22,23}. These changes follow a clear temporal and spatial pattern with rapid up-regulation of certain molecules (e.g. P and E selectin); others are constitutively expressed but activation up-regulates their function (e.g. integrins). Generally, changes in adhesion molecule expression are restricted to the region of inflammation and specific adhesion molecules may be invoked in specific tissues such as MAdCAM-1 expression for lymphocyte trafficking in the gastrointestinal tract (GIT).

Classically, leukocyte adhesion is described in terms of rolling, firm adhesion and transmigration. The selectins – E-selectin (CD62E), P-selectin (CD62P) and L-selectin (CD62L) – mediate the low affinity binding of neutrophils and monocytes to the endothelium that allows rolling\textsuperscript{24}. There is evidence that other molecules, including CD40, can also participate in this process. The vascular selectins (E and P-selectin) are rapidly up regulated by a number of agents (TNFα, IL-1β IFN-γ, thrombin and LPS). By contrast, L-selectin is constitutively expressed on leukocytes and activation can lead to shedding of this molecule. Through interactions with their glycoprotein ligands (sialyl Lewis-X, peripheral lymph node addressin) the selectins bring leukocytes into temporary contact with the endothelium which allows firm adhesion interactions – mediated by integrin-immunoglobulin superfamily interactions – to occur. The β\textsubscript{2} integrins are the main leukocyte integrins involved although many other integrins participate, especially PECAM (CD31) and the β\textsubscript{1} integrin VLA-4 (Cd29/CD49d). The β\textsubscript{2} integrins are heterodimers with a common β\textsubscript{2} chain (CD18) linked to one of three α chains (CD11a, CD11b and CD11c) to form LFA-1, Mac-1 and p150/95, respectively. The major endothelial ligands for these β\textsubscript{2} integrins are ICAM-1 (CD54), ICAM-2 (CD102) and VCAM-1 (CD106). ICAM-1 is upregulated by most activating agents including hypoxia while ICAM-2 is constitutively expressed on endothelial cells. ICAM-1 binds to all three β\textsubscript{2} integrins in addition to rhinovirus and Plasmodium falciparum infected red blood cells, ICAM-2 binds to LFA-1, VCAM-1 binds to VLA-4 and PECAM binds other PECAM molecules homotypically; both PECAM and VLA-4 are essential for transmigration\textsuperscript{25}. Unlike most of the immunoglobulin superfamily (e.g. ICAM-1 and VCAM-1 where activation significantly increases surface expression), many of the leukocyte integrins are constitutively expressed and activation markedly increases their function rather than level of expression (Fig. 3)\textsuperscript{26}.

Those rare patients with leukocyte adhesion molecule deficiency syndromes demonstrate the importance of the adhesion molecules in modulating normal inflammatory processes most clearly. Deficiency of CD18 leads to a loss of integrin-mediated adhesion and inability of leukocytes to extravasate. These patients are unable to respond to bacterial activating signals and, despite high circulating numbers of neutrophils, they suffer from recurrent severe infections\textsuperscript{27}. Blocking
adhesion molecules in animal models of overwhelming inflammation prevents inflammatory cell influx and can reduce mortality. In a similar fashion, antibodies against adhesion molecules can prevent lethal injury in ischaemia-reperfusion models. However, making a transition from a pre-treatment that blocks excessive inflammatory cell adhesion in a clearly defined model to clinical applications in which the inflammatory cascade is already activated remains a major challenge.

**Phagocytosis and killing**

Activation increases the phagocytic ability of inflammatory cells, partly due to increased expression of the complement and other receptors and partly to mobilisation of cytoskeletal elements. Once phagocytosed, fusion with intracellular granules exposes micro-organisms to bactericidal agents, including histotoxic enzymes and ROI (superoxide, hydrogen peroxide and hypochlorous acid). These free radicals are highly reactive species produced with activation; indeed, the production of superoxide is the gold standard activation response of neutrophils and requires a massive increase in oxygen consumption, called the
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respiratory burst. Activation leads to assembly of the NADPH oxidase system which catalyses the following reaction:\(^{28}\):

\[
\text{NADPH} + \text{H}^+ + 2\text{O}_2 = \text{NADP}^+ + 2\text{H}^+ + 2\text{O}_2^-
\]

Superoxide dismutase then converts superoxide to hydrogen peroxide and myeloperoxidase catalyses the conversion of hydrogen peroxide into hypohalous acids, especially hypochlorous acid. As with each facet of the activation/inflammatory cascade, the ability to augment ROI production rapidly is vital to normal host defence, but an excessive response is damaging\(^4\). Patients with chronic granulomatous disease are unable to generate ROI species due to defects in their NADPH oxidase system. They suffer from recurrent staphylococcal and fungal infections and have a reduced life expectancy. Overproduction of ROI is just as damaging, with increased levels found in numerous clinical conditions, common in the critically ill, such as reperfusion injury of the heart, lung or brain; smoke inhalational injury and alveolar damage in patients with ARDS. Furthermore, mechanical ventilation may exacerbate this process as exposure to 100% oxygen has been calculated to increase mitochondrial hydrogen peroxide production up to 15-fold. There is now compelling data in animal models supporting the use of anti-oxidant therapy in many conditions such as trauma resuscitation and in the treatment of shock\(^4\). A number of different approaches exist, including augmenting endogenous anti-oxidant mechanisms with superoxide dismutase or glutathione, or blocking free radical production using agents such as allopurinol. Other potentially useful applications include the use of desferrioxamine, which chelates iron and blocks the Fenton reaction or the provision of anti-oxidant vitamins or 21-aminosteroids. All these therapies have yet to make the transition to effective clinical strategies\(^{29}\).

Secretion of mediators

Chemotaxins

Migration into an inflammatory focus requires not only appropriate adhesion molecule expression but also a chemotactic gradient. Many molecules have chemotactic properties (Table 2). Perhaps the best example is IL-8, a potent neutrophil priming and chemotactic agent secreted by alveolar macrophages amongst other cells. The clinical significance of this is demonstrated by the finding of high levels of IL-8 only in the broncho-alveolar lavage fluid of those patients destined to later develop full blown ARDS but not in those equally at risk but who do not develop lung problems\(^{30}\).
Table 2 Some agents chemotactic for inflammatory cells

<table>
<thead>
<tr>
<th>Plasma derived factors</th>
<th>Complement products: C5a and C5a des arg</th>
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<tr>
<td></td>
<td>Fibrinopeptides</td>
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<td>IgG proteolytic fragments</td>
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<tr>
<td>Cell derived factors 1</td>
<td>Complement products: C5a and C5a des arg</td>
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<td></td>
<td>Fibrinopeptides</td>
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<td>IgG proteolytic fragments</td>
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<tr>
<td>Cell derived factors 2</td>
<td>(Leukotriene B4) LTB4</td>
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<tr>
<td></td>
<td>Platelet factor-4</td>
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<tr>
<td>Cytokines and chemokines</td>
<td>TNFα</td>
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<tr>
<td></td>
<td>IL-1</td>
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<td>IFN-γ</td>
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<td>IL-8</td>
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<td></td>
<td>(Macrophage inflammatory protein-1 alpha) MIP-1α</td>
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<td></td>
<td>(Macrophage chemoattractant protein-1) MCP-1</td>
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<tr>
<td>Bacterial derived factors</td>
<td>LPS</td>
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<td></td>
<td>fMLP</td>
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Enzymes

Activation of inflammatory cells leads to degranulation and release of the numerous potentially damaging enzymes, including elastase, myeloperoxidase, neutral proteases, the acid hydrolases, collagenase and plasminogen activator. The intimate contact between inflammatory cells and endothelium promoted by leukocyte plugging and adhesion ensures these substances can be released into a protected micro-environment. Here they are immune to one arm of the CARS response, the protective effects of circulating antiproteases. Antiproteases such as α1 antitrypsin, plasminogen activator inhibitor and α2 macroglobulin (which inhibits further protease secretion) are key anti-inflammatory mediators and, in such a protected micro-environment, degranulation products can cause extensive endothelial damage, potentiating capillary leak and cellular extravasation. There is now evidence that increased levels of these histotoxic agents exist in hyper-inflammatory states (e.g. enhanced levels of neutrophil elastase and collagenase in ARDS).

Lipid mediators

Activation also promotes the synthesis and release of multiple short lived lipid mediators of inflammation including PAF, thromboxane A2, LTB4, and other leukotrienes from stimulated neutrophils and macrophages. These powerful agents prime and activate inflammatory cells in addition to their multiple other actions, including their effects on vasomotor and bronchial muscle tone and on platelet function and capillary permeability.
Although recent evidence suggests that prostaglandin E₁ may attenuate neutrophil cytotoxic mechanisms, excessive prostanoid synthesis is detrimental. This is regulated by the cyclo-oxygenase (COX) enzymes, which are inhibited by non-steroidal anti-inflammatory drugs including aspirin. Inflammatory cells express COX-2, the inducible cyclo-oxygenase isoform, in response to LPS, TNFα and IL-1β in parallel with PGE₂ generation. There is now much interest in selective modulation of COX-2 to provide a strategy that may break the positive feedback loop whereby activation induces icosanoid production that induces further activation.

**Cell lifespan**

The lifespan of most inflammatory cells is relatively short in the absence of any activating stimuli. Neutrophils normally only survive a matter of hours *in vitro* before dying by apoptosis and being cleared by phagocytes. A number of agents including the colony stimulating factors, granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) and pro-inflammatory cytokines such as TNFα act to prolong the normal neutrophil life-span. G-CSF is now widely used to overcome neutropenias of varying origins. Monocytes also die by apoptosis in the absence of activating stimuli and a similar range of cytokines potentiates their survival. Inflammatory mediators can, therefore, promote inflammatory cell function by prolonging survival, although their excessive persistence is detrimental. Macrophages remove apoptotic cells by phagocytosis, employing specific receptors including CD36 and the integrin αvβ3 and also probably the scavenger receptor for this. This mechanism is specific for apoptotic cells and does not invoke further activation signals from the macrophage. Macrophages themselves are cleared by active emigration to the draining lymph nodes, a process that may well depend upon their activation state. Thus, not only do pro-inflammatory signals increase the functional capacity of inflammatory cells but they also prolong their lifespan and are directly relevant to the resolution of inflammation.

**Activation of neutrophils and macrophages: the differences**

The main differences between activation of neutrophils and macrophages relate to their different functions. Neutrophils engulf and kill pathogens, whilst macrophages have a far greater range of functions. Like neutrophils, they recognise and remove inflammatory stimuli and secrete mediators, but the wealth of their secretory repertoire is far greater and
includes enzymes, lipid mediators, complement, coagulation mediators, matrix components, cytokines and colony stimulating factors. Macrophages also present antigen, invoke an immune response, promote the resolution of inflammation by tissue debridement and stimulation of fibrosis and angiogenesis. Thus, macrophages can be regarded as generals co-ordinating an army of neutrophils and other activated cells.

Although the activation signals are similar for both cells, their responses differ. Many adhesion molecules are common to both but important differences exist, reflected in different kinetics. Thus, a rapid neutrophil influx is seen in response to an inciting stimulus that soon ceases in contrast to the delayed but persistent influx of monocytes3,37,38.

Neutrophils and macrophages also differ in their response to chemo-tactic agents. For example, collagen type 1 and fibronectin proteolytic fragments are specifically chemotactic for macrophages, whilst IL-8 is specific for neutrophils. Moreover, while C5a and C5a des arg are equally chemotactic for both, conversion from C5a into C5a des arg reduces the neutrophil response alone.

Further contrasts exist in cellular enzyme capacity. Neutrophils contain myeloperoxidase (MPO) which is absent in macrophages. With immuno-activation macrophages, unlike neutrophils, express the inducible form of nitric oxide synthase, iNOS or NOS2 which is closely linked to their antimicrobial capacity39,40. Critically ill patients have increased nitric oxide (NO) production with elevated nitrite and nitrate levels. NO can exert a regulatory effect on neutrophil responses by reducing Mac-1 expression and limiting NADPH oxidase mediated superoxide production41. Despite these beneficial actions, excessive NO generation may contribute to the vascular paresis that characterises septic shock. Furthermore, NO reacts in water with oxygen to yield other radical species including the highly unstable peroxynitrate (ONOO−) leading to lipid peroxidation and extensive cell damage. Moreover, NO is a competitive inhibitor of the electron transfer chain and can block the cell’s capacity to consume oxygen. Septic shock differs from other causes of shock in that, although delivery of oxygen to the tissues is increased, oxygen extraction by the tissues is reduced, with features of anaerobic respiration including metabolic acidosis and hyperlactaemia. There is now evidence that LPS mediated production of NO contributes to this defect42.

**Specialised sites**

Certain sites are at particular risk from diffuse inflammatory cell activation. Thus, the lung is damaged after many forms of insult at distant sites. Studies using radiolabelled cells demonstrate neutrophil accumulation within the lung in patients with ARDS, which is
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dependent, in part, upon the unique circulation of the lung, all leukocytes having to pass through the pulmonary microvasculature. Activation leads to neutrophil cytoskeletal actin assembly resulting in cell shape change and altered rheology, the associated reduction in cell deformability limits its ability to squeeze through narrow capillaries, leading to plugging which is enhanced by increased adhesion molecule expression. Specific cellular activation factors also recruit leukocytes to the lungs at a very early stage in ARDS and in retain macrophages within this site\textsuperscript{30}.

The GIT is another organ worth special consideration as it carries an enormous microbiological load within its lumen that normally it maintains effectively separated from the body. The anatomy of the microcirculation of the gut, with end arteries (which branch off at right angles from their own feeding arteries) supplying the villi within the intestine, mean that when the general circulation is compromised these vessels may not be able to maintain an adequate blood supply. This has led to the hypothesis that critical illness and hypoperfusion can lead to loss of the GIT mucosal barrier with the consequent potential of a leak of bacteria and their products, in particular endotoxin from the gut lumen into the circulation, further driving the inflammatory processes\textsuperscript{10}. Certainly, increased permeability of the gut mucosa has been demonstrated in response to many different challenges, but the relevance of this to bacterial translocation has not been established. Furthermore, although studies in animal models of circulatory shock have confirmed the presence bacteria in portal blood and mesenteric lymph nodes, no causal link between a precipitating illness, bacterial translocation and remote organ injury has been established in man\textsuperscript{10}.

**Downregulating effects**

Many endogenous controls on the inflammatory cascade exist as part of the CARS. Specific cytokines down-regulate many of the functions of activated inflammatory cells. For example, IL-10 when given prior to inflammatory challenge in animal models inhibits LPS induced release of TNF\textalpha, IL-1\beta, IL-6 and IL-8 and reduces mortality. IL-4 inhibits LPS induced COX-2 expression and reduces macrophage responses\textsuperscript{43}. Other protective molecules include the antiproteases and specific proteins that can block exogenous activating agents; notably bactericidal permeability increasing protein BPI, a weak antibacterial protein found in neutrophil granules. BPI binds LPS avidly and may play a powerful role as an ‘endotoxin scavenger’ within the neutrophil itself, preventing the systemic release of LPS after intracellular bacterial killing and thus limiting cellular activation\textsuperscript{44}. The scavenger receptor type A has a similar
capability. The expression of this trimeric glycoprotein is upregulated with macrophage activation and it binds to both LPS and lipoteichoic acid. Increased scavenger receptor expression limits TNFα secretion and protects mice from lethal endotoxaemia.

The body synthesises protective endogenous anti-oxidants including superoxide dismutase, catalase and glutathione peroxidase, the levels of which may be deficient in certain states including the premature infant and in children with cyanotic congenital heart disease. Another regulatory protein is the intracellular redox regulating molecule thioredoxin, a stress inducible protein that protects cells from oxidative stress induced apoptosis.

In addition to these protective molecules, many surface receptors are shed upon activation including cytokine receptors (e.g. both TNF receptors, IL-6 and M-CSF receptors) adhesion molecules (e.g. ICAM-1, VCAM-1 and L-selectin) and others including CD14. Some, such as the IL-6 receptor and CD14 activate inflammatory cells when shed, whilst others, such as sICAM-1 and soluble TNF receptors exert negative regulatory effects. IL-1 is regulated by the endogenous IL-1 receptor antagonist IL-1ra, found in increased levels in febrile patients. Thus, it is the balance of pro- and anti-inflammatory cytokines that may be more important than the absolute levels of any single cytokine and there is evidence for this in that the ratio of IL-1β to IL-1ra favours unopposed inflammation in established ARDS.

**Current and future therapeutic options**

Many drugs in clinical use modulate the activation of inflammatory cells. The best examples of this are the glucocorticoids which in vitro reduce the neutrophil respiratory burst, prevent neutrophil death and modulate intracellular signal transduction pathways, blocking the release of pro-inflammatory cytokines. Pretreatment in animal models in vivo halves neutrophil influx into inflammatory sites. Steroids are, however, harmful to patients in the general treatment of sepsis, although they can be beneficial in the face of overwhelming septic shock or if used later in the inflammatory process, especially for ARDS.

Many other drugs routinely used in critical illness modulate the inflammatory process; for example, non-steroidal anti-inflammatory drugs and H2 blockers have been implicated as immunosuppressants. Morphine also reduces macrophage phagocytic ability, chemotactic responses and ROI production and NK, T and B cell responses. Similarly, the intravenous anaesthetic propofol inhibits LPS induced IL-8 release and dopamine inhibits prolactin release and may contribute to the anergy seen in critical illness.
Table 3 Areas for potential therapeutic intervention in the future

<table>
<thead>
<tr>
<th>The future</th>
<th>Potentially successful strategies</th>
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<tr>
<td>1. Develop better animal models to</td>
<td>Model specific infections or injuries not bolus LPS injections</td>
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<tr>
<td>Mimic specific clinical situations accurately</td>
<td>5–14 day models</td>
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<tr>
<td>Monitor long term consequences</td>
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<tr>
<td>2. Develop tests reflecting the immuno-inflammatory state</td>
<td>Responsiveness of leukocytes to challenge</td>
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<tr>
<td>Determine if hyper or hypo-responsive state exists</td>
<td>Adhesion molecule expression</td>
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<td></td>
<td>Balance of pro- and anti-inflammatory cytokines</td>
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<td></td>
<td>HLA-DR expression on monocytes</td>
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<tr>
<td>3. Develop therapies to appropriately</td>
<td>Combined endogenous anti-inflammatory agents:</td>
</tr>
<tr>
<td>Limit hyper-responsive pro-inflammatory states</td>
<td>(bactericidal permeability increasing factor (BPI), IL-1ra, IL-10, IL-13)</td>
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<tr>
<td></td>
<td>Anti-oxidant therapy</td>
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<tr>
<td>Boost hypo-responsive inflammatory states</td>
<td>IFN-γ</td>
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Experimental evidence from models of sepsis suggests that therapies aimed at limiting the activation pathways of inflammatory cells should prevent the deleterious consequences of overstimulation. As discussed elsewhere, these hopes have not yet been translated into clinical reality. The reasons for this are multifactorial, but a fundamental problem lies in the animal models currently employed to examine the mechanisms and treatment of sepsis and inflammation. Difficulties include species differences, inappropriate experimental design and poor relationship between models of inflammation and the clinical states being treated. Models need to be developed wherein inflammation is already established before therapeutic intervention, this inflammation is induced by a clinically relevant mechanism and the model can be monitored long term with appropriate resuscitation to accurately establish mechanisms and mortality (Table 3).

Despite the failures of current immunotherapeutic approaches this review highlights that, should methods be developed to limit but not ablate activation at the appropriate time in the inflammatory process, they are likely to yield significant clinical benefits. The reader will further understand that critical illness can also lead to states of hypo-responsiveness whereby augmentation of the inflammatory process would be beneficial and therapies aimed at activation would be required in these circumstances. This requires the ability to assess rapidly and accurately the immuno-inflammatory state of the patient to decide when and if pro- or anti-inflammatory measures should be initiated. Of the
many potential activation pathways discussed, ones that may hold out more promise in the near future include the use of anti-oxidants and BPI to regulate excessive inflammatory activation and the use of IFN-γ and possibly G-CSF as immunostimulants in the case of hypo-responsiveness. It is likely that combination therapy will be more effective due to the huge redundancy within the inflammatory response.

Key points for clinical practice

1. The body depends upon the rapid activation of inflammatory cells for an effective inflammatory response.

2. Overstimulation of the inflammatory response is damaging. However, impaired activation (neutropenia, congenital disorders or more commonly as a consequence of prolonged severe inflammation) reduces the ability of the body to defend itself against infection.

3. There is enormous redundancy in the inflammatory processes. A single mediator alone is unlikely to regulate the whole inflammatory process.

4. Although the use of animal models has led to a far greater understanding of the mechanisms underlying inflammation, they have not yet successfully translated to the treatment of the human condition of systemic inflammation. This will depend upon the development of more relevant models, more accurately representing the initiation and progress of critical illness.

5. The therapeutic goal remains to modulate inflammatory cell activation at the correct time during the course of critical illness so as to maintain a balanced response. This depends on a clear understanding of the underlying pathophysiological processes and evidence demonstrating a hypo or hyper-responsive state.

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