The immuno-inflammatory cascade

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Severe sepsis complicated by multi-organ dysfunction syndrome (MODS) is a major cause of death in intensive care units, with a mortality rate in excess of 50%. The outcome is determined not only by the infection but also by the intensity of the immuno-inflammatory response. This response is essential for the resolution of infection but may occur in an uncontrolled manner causing damage to the host. The pronounced synergy and interaction of the components of the immune system dictate that modulation may result in either immunostimulation or immunosuppression. Coordination of the host immune response to infection and inflammation in terms of expression of the effector molecules is vital to an optimum response (Fig. 1).

Innate and acquired immunity

Mediators of immunity and inflammation (families of protein and lipid molecules) are part of an intercellular signalling language which allows cells/tissues/organs to take in new information and, based on past experience, decide what to do next. There are essentially two components to the immune response—in innate (non-specific) and acquired (antibody-mediated) immunity.

The body possesses a range of barriers to prevent micro-organisms from entering, including the skin, mucous secretion, ciliary action and gastric acid. If these barriers are crossed micro-organisms are destroyed by soluble factors such as lysozyme and by phagocytosis with intracellular digestion (termed innate immunity). The complement system is a multi-component triggered enzyme cascade which attracts phagocytes to micro-organisms increasing capillary permeability and neutrophil chemotaxis and adhesion. Specific acquired immunity in the form of antibodies inactivates micro-organisms which are not destroyed by the innate immune system. Such micro-organisms either fail to activate the complement pathway or prevent activation of phagocytes. The cells involved in innate immunity include “professional” phagocytes (polymorphonuclear neutrophils, mast cells and macrophages) and “non-professional” phagocytes (endothelial cells and hepatocytes). Cells infected with viruses and parasites are killed by large granular lymphocytes termed natural killer (NK) cells, and eosinophils.

Acquired immune defences against specific micro-organisms (antigens) form the second component of the immune response. Antibodies activate the complement system, stimulate phagocytic cells and specifically inactivate micro-organisms. Lymphocytes, the basis of the acquired immune defence system, consist of antibody-producing plasma cells derived from B-lymphocytes, and T-lymphocytes which control intracellular infections. Binding of micro-organisms to antibodies on the cell surface of B-cells leads to preferential selection of these antibody-producing cells. This is termed priming, and subsequent responses are faster and amplified, and provide the basis of vaccination. T-cells exploit two main strategies to combat intracellular infections—secretion of soluble mediators which activate other cells to enhance microbial defence mechanisms, and production of cytolytic T-cells which kill the target organism. Adaptive selection of specific T-cell subsets occurs in response to local balance of cytokine concentrations [12].

Cytokines

Orchestration of immune and inflammatory responses depends upon communication between cells by soluble molecules given the generic term cytokines, including chemokines, interleukins (IL), growth factors and interferons (IFN). They are low molecular weight secreted proteins which regulate both the amplitude and duration of the immune/inflammatory responses (table 1). They have a transient action which is tightly regulated. Cytokines are highly active at very low concentrations, combining with small numbers of high affinity cell surface receptors and producing changes in RNA and protein synthesis. They have multiple effects on growth and differentiation in a variety of cell types with considerable overlap and redundancy between them, partially accounted for by the induction of synthesis of common proteins. Interaction may occur in a cascade system in which one cytokine induces another, through modulation of the receptor of another cytokine and through either synergism or antagonism of two cytokines.
Acting on the same cell, cytokines should not be considered as having identifying labels for being growth stimulators or inhibitors and pro- or anti-inflammatory actions. Their specific actions depend on the stimulus, the cell type and the presence of other mediators and receptors.

Chemokines are a family of small, pro-inflammatory molecules characterized by four conserved cysteine residues. The α-chemokines have two pairs of cysteine residues separated by a variable amino acid and chemoattract neutrophils (e.g. interleukin 8, platelet basic protein, epithelial neutrophil activating peptide) whereas β-chemokines have two adjacent pairs of cysteine groups and are chemoattractant for monocytes/macrophages (e.g. platelet factor 4, monocyte chemotactic protein 1, macrophage inflammatory protein 1) and T-cells (e.g. RANTES). Chemokines have been described as having more restricted actions than cytokines, but this is more likely to be the result of differential expression of receptors [9]. Interferons (IFN α, β, γ) are a family of broad spectrum antiviral agents which also modulate the activity of other cells, particularly IL-8 and platelet activating factor (PAF) production, antibody production by B-cells and activation of cytotoxic macrophages [5]. Growth factors regulate the differentiation, proliferation, activity and function of specific cell types [17]. The best known are colony stimulating factors which cause colony formation by haematogenic progenitor cells (e.g. granulocyte-macrophage colony stimulating factor or GM-CSF). Other examples include factors which regulate the growth of nerve cells, fibroblasts, epidermis and hepatocytes.

In addition to the low molecular weight protein mediators, there are also lipid mediators of inflammation which include PAF and arachidonic acid metabolites. Platelet activating factor is a labile alkyl phospholipid released from a variety of cells in the presence of antigen and leucocytes in response to immune complexes. In addition to its platelet effects, the actions of PAF include the priming of macrophages to other inflammatory mediators and alterations of microvascular permeability [11]. Arachidonic acid metabolites include the prostaglandins, leukotrienes, HETE and HPETE, all of which have profound inflammatory and vascular actions, and may regulate and be regulated by, other cytokines.

Tumour necrosis factors (TNF) α and β have a vast range of similar effects and are usually referred to as inflammatory cytokines [2]. They have a central role in initiating the cascade of other cytokines and...
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Factors that make up the immune response to infection. The wide variety of effects is attributable to the ubiquity of their receptors, their ability to activate multiple signal transduction pathways and their ability to induce or suppress an array of genes including those for growth factors, cytokines, transcription factors, receptors and acute phase proteins. Although both TNFs have similar biological activities, regulation of the expression and processing of the two is quite different.

Control of adaptive T-cell selection

The major histocompatibility complex (MHC) includes genes encoding class I and II cell surface glycoproteins whose function is to present antigenic peptides to T-cells. Regulation of MHC gene expression, e.g. by cytokines (table 1), plays a fundamental role in the immune system, since alterations of cell surface expression of class I or II molecules can affect the efficiency of antigen presentation. T-lymphocytes consist of two subsets: T helper (Th)-cells which recognize class II MHC molecules and which produce IFNγ and other macrophage-activating factors; cytotoxic or killer T-cells which recognize both specific antigens and class I MHC molecules on the surface of infected cells.

Circulating Th-cells are capable of unrestricted cytokine expression and are prompted into a more restricted and focused pattern of cytokine production depending on signals received at the outset of infection [12]. Th-cells can be classified according to the pattern of cytokines they produce. Th1 category cells secrete a characteristic set of cytokines which push the system towards cellular immunity (cellular cytoxicity). Th2-cells are associated with humoral or antibody-mediated immunity. Typically Th1 cells secrete IL-2, IFNγ, TNFβ and transforming growth factor β (TGFβ) whereas the Th2-cells secrete IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 and also help B-cell antibody production. Both cell types produce IL-3, TNFα and GM-CSF. Interleukins 12 and 4 have been identified as early inducers of Th1 and Th2 responses respectively, and therefore the local balance of these cytokines is an important determinant of subsequent immune responses. Understanding the influence of antigen-independent cytokine production on the subsequent acquisition of adequate immune responses to an infection is clearly important.

Receptors and antagonists

The biological activities of cytokines are regulated by specific cellular receptors. Often these receptors comprise multiple subunits providing phased stages of activation and biological action. For example, the IL-2 receptor complex consists of three subunits, IL-2Rα, IL-2Rγ and IL-2γ. Although the IL-2Rα/β combination can bind IL-2, IL-2Rγ is also required for high affinity binding, ligand internalization and signalling which are required for maximal effect [14]. Other cellular receptors exist in more than one type which act alone but have different binding affinities for different forms of a cytokine protein (e.g. IL-1 receptor type I binds IL-α better than IL-1β, and IL-1 receptor type II has more affinity for IL-β). Binding of a cytokine to one type of receptor may result in interactions with another receptor; the two receptors for TNF, for example, use ligand passing in which TNF binds transiently to receptor type I, with full signal transduction, but may then move onto the type II receptor with activation of another signal for apoptosis or programmed cell killing [20].

Soluble cytokine receptors have been identified which compete with membrane-bound receptors, thus regulating cytokine signals. Exceptions to this are soluble receptors for IL-6 and ciliary neurotrophic factor which act as agonists rather than antagonists [19]. Such soluble receptors may be membrane-bound receptors which are shed into the circulation either intact or as truncated forms (e.g. soluble TNF receptors, sTNF-R), or may begin as related precursor molecules which are enzymatically cleaved (e.g. IL-1R). Soluble receptors may appear in response to stimuli as part of a naturally occurring independent regulatory process to limit the deleterious effects of a mediator (e.g. sTNF-R), but some soluble receptors have little binding activity and may represent superficial and unimportant losses of cellular receptors (e.g. the soluble form of the IL-2Rα). Soluble cytokine receptors not only mediate biological activity but control desensitization to ligands by reduced availability, decreased signalling and by stimulating cellular mechanisms which can result in lack of activity.

The biological actions of some cytokines are also regulated by receptor antagonists. The receptor antagonist for IL-1 (IL-1ra) competes with cell receptors for IL-1, but when bound does not induce signalling. IL-1ra binds to cell receptors much more avidly than to soluble receptors, such that soluble receptors will have little effect of the inhibitory action of the receptor antagonist. The soluble receptor also inhibits activation of the pro-IL-1β precursor. The appearance of IL-1ra is independently regulated by other cytokines as part of the inflammatory process [4].

Mechanisms of multi-organ failure

Severe infection leads to the appearance of endotoxin or lipopolysaccharide (LPS) in the bloodstream which triggers the innate immune responses such as activation of phagocytic cells and activation of the alternative complement cascade, leading to the production of the primary pro-inflammatory mediators, TNF and IL-1. Secondary mediators including other cytokines, prostaglandins and PAF are then released, with further activation of complement and the acute phase response, expression of adhesion molecules, T-cell selection, antibody production and release of oxygen-derived radicals [3, 7, 21]. Other toxins and cellular debris must also trigger such a systemic inflammatory response, since this process occurs in the absence of LPS release.

Local effects of the inflammatory response are essential for the control of infection. Prolonged systemic exposure to high concentrations of cyto-
**Table 1** Sources and biological effects of the immune mediators (ARDS, acute respiratory distress syndrome; GM-CSF, granulocyte-macrophage colony stimulating factor; G-CSF, granulocyte-colony stimulating factor; IFN, interferon; IgA, immunoglobulin A; IgG, immunoglobulin G; IL, interleukin; IL-1ra, interleukin 1 receptor antagonist; MHC, major histocompatibility complex; mRNA, messenger RNA; NK, natural killer cells; NO, nitric oxide; NOS, nitric oxide synthase; ODFR, oxygen-derived free radicals; PAF, platelet activating factor; Pg, prostaglandin; TGF, transforming growth factor; TNF, tumour necrosis factor.)

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Source</th>
<th>Biology activity</th>
<th>Effects on other cells</th>
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<tbody>
<tr>
<td>IFNα</td>
<td>T-cells</td>
<td>Pyrogenic, cytotoxic.</td>
<td>Macrophages (increases class I MHC antigens, IL-1, PAF production); B-cells (proliferation, differentiation); T-cells (proliferation); chemotactic.</td>
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<td>IFNβ</td>
<td>B-cells, Macrophages, Fibroblasts</td>
<td>Pyrogenic; antiviral; cytotoxic; anti-tumour effect; mimics septic shock; causes release of NO and ODFRs; and upregulates IL-1 and PAF production</td>
<td>Macrophages (increases class I MHC antigens, IL-1, PAF production, downregulates IL-2 mediated IL-8 mRNA); B-cells (proliferation and differentiation); chemotactic for monocytes; stimulates formation of adhesion molecules.</td>
</tr>
<tr>
<td>IFNγ</td>
<td>T-cells, NK cells</td>
<td>Pyrogenic; antitumour effect; mimics septic shock; causes release of ODFRs</td>
<td>Wide variety of effects due to ability to mediate expression of genes. Important role in host resistance to infection as immunostimulant and mediator of the inflammatory response. Promotes haematoipoiesis. Important role in intercellular communication.</td>
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<tr>
<td>TNFα</td>
<td>Neutrophils, Lymphocytes, NK cells, Endothelial cells, Smooth muscle cells</td>
<td>Pyrogenic; cytotoxic; anti-tumour effect; mimics septic shock; promotes angiogenesis; causes release of NO and ODFRs; induces or suppresses gene expression for cytokines, receptors and acute phase proteins.</td>
<td>Macrophages (TNF and IL-6 production); B-cells (proliferation, differentiation; T-cells (proliferation) chemotaxis; formation of adhesion molecules; haematoipoiesis.</td>
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<tr>
<td>IL-1</td>
<td>Macrophages, Endothelial cells, Fibroblasts, Hepatocytes</td>
<td>Pyrogenic; cytotoxic; anti-tumour effect; promotes angiogenesis; causes release of NO and ODFRs; induces prostaglandin synthesis; initiates the acute phase response.</td>
<td>Macrophages (TNF and IL-6 production); B-cells (proliferation, differentiation, release of IgG from activated B-cells); chemotaxis; augments neutrophil and macrophage function; formation of adhesion molecules.</td>
</tr>
<tr>
<td>IL-2</td>
<td>T-cells</td>
<td>Pyrogenic; anti-tumour effect; mimics septic shock; causes release of ODFRs.</td>
<td>B-cells and T-cells (proliferation, differentiation, release of IgG from activated B-cells); chemotaxis; augments neutrophil and macrophage function; formation of adhesion molecules.</td>
</tr>
<tr>
<td>IL-4</td>
<td>T-cells, B-cells, Macrophages</td>
<td>Cytotoxic; anti-tumour effect; inhibits induction of nitric oxide synthase; inhibits release of superoxide by macrophages; numerous anti-inflammatory effects.</td>
<td>Macrophages (suppresses activation, upregulates class II MHC antigens, inhibits IgG receptor expression, inhibits expression of IL-1, IL-6, IL-8, TNF, stimulates IL-1ra expression); B-cells and T-cells (proliferation, differentiation, enhances antigen-presenting capacity); chemotaxis; formation of endothelial cell adhesion molecules; haematoipoiesis.</td>
</tr>
<tr>
<td>IL-6</td>
<td>T-cells, Macrophages, Endothelial cells, Fibroblasts, Hepatocytes</td>
<td>Cytotoxic; anti-tumour effect; mimics septic shock; causes release of ODFRs; induces hepatic acute phase proteins</td>
<td>B-cells (differentiation, antibody production); T-cells (activation, proliferation, differentiation, induces IL-2 production); formation of adhesion molecules; haematoipoiesis.</td>
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Kines and other components of the immunoinflammatory cascade may contribute to the development of MODS. Damage and activation of the endothelium, which plays a pivotal role in the regulation of haemostasis, vascular tone and fibrinolysis, have profound consequences. The endothelium produces several substances which regulate inflammation and regional perfusion, including nitric oxide, vasoactive arachidonic acid metabolites and cytokines [18]. Changes in the balance of concentrations of these substances may contribute to the pathogenesis of the inflammatory response during sepsis and injury. Phagocytic cells are in constant contact with the endothelium and disturbance of the relationship between these two cell types may result in direct tissue damage as a result of local production of oxygen-derived free radicals, hypochlorous acid and proteolytic enzymes. Another hypothesis to explain the observed tissue damage and organ dysfunction is that of local tissue ischaemia and hypoxia as a result of microthrombi formed by a coagulopathy or platelet or white cell aggregates. There is evidence for all of these mechanisms and it is probable that the pathogenesis of MODS and organ failure is diverse and complex and is unlikely to be attributable to a single mechanism.

**Potential for therapy**

Both soluble receptors and monoclonal antibodies directed against receptors can be used to block the interaction of a cytokine with its receptor. This then prevents transduction of the appropriate biological signal in the target cell. The cloning of genes encoding cytokine receptor chains and the characterization of their soluble forms has resulted in new approaches to anti-cytokine therapy. Injection of a recombinant soluble receptor might prevent the deleterious effect of excessive cytokine production. In addition to soluble receptors, monoclonal antibodies which block cellular cytokine receptors can be used as anti-cytokine therapy. However, these small
molecules have short half-lives and therefore derivatized molecules with longer half-lives and higher affinity have now been developed. However, it has been shown that cytokine complexed to such binding proteins is still available for receptor binding [10]. It is possible that these complexes can still act as agonists in vivo depending on concentrations of other mediators and relative receptor expression. Another approach to minimizing the deleterious effects of the uncontrolled inflammatory process is to blunt the final common pathways of damage (i.e. using either agents which decrease free radical production or antioxidants which inactivate free radicals as they are produced).

Monoclonal antibodies to TNFs have been used both in clinical and animal studies [22]. The use of sTNF receptors has also been evaluated in sepsis [1, 23]. The naturally occurring IL-1 receptor antagonist has been studied in two large clinical trials [15]. It is also possible to modulate the production of TNF at the mRNA level using pentoxiphylline, and there have been several promising animal studies [8]. Specific chemical antagonists, for example against PAF, have also been evaluated in patients with sepsis.

Blockade of any single or combined inflammatory mediator may not be successful for a number of reasons. First, the immuno-inflammatory process is a normal response to infection and is essential not only for the resolution of infection but also for the initiation of other adaptive stress responses required for host survival (e.g. acute phase and heat shock responses) [16]. Second, the profound redundancy of action of many cytokines means that there are many overlapping pathways for cellular activation and further mediator release. In addition, the synergism of actions and effects of many cytokines suggests that imbalance in the process of the immune response may be adversely affected by inhibition of a single agent. We also suggest that exogenously administered anti-cytokine therapy may have hitherto unrecognized effects caused by interaction with

### Table 1

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<tr>
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<tbody>
<tr>
<td>IL-8</td>
<td>T-cells Macrophages Endothelial cells Hepatocytes Neutrophils Fibroblasts</td>
<td>Angiogenic; leucocyte infiltration in septic shock and ARDS.</td>
<td>Neutrophils (activation); upregulates cell adhesion molecules; chemotactic for PMNs.</td>
</tr>
<tr>
<td>IL-10</td>
<td>T-cells B-cells Macrophages</td>
<td>Inhibits induction of nitric oxide synthase; suppresses synthesis of ODFRs; may be immunostimulatory or immunosuppressive.</td>
<td>Macrophages antigen presenting capacity, downregulates class II MHC antigen expression, suppresses PGE2, TNF, IL-1, IL-6, IL-8 production; B-cells (induces IgA synthesis, enhances survival, upregulates IL-2 receptors); T-cells (inhibits IFNγ); neutrophils (inhibits pro-inflammatory cytokine synthesis, upregulates IL-1ra expression).</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Macrophages Endothelial cells</td>
<td>Proliferation, differentiation and activation of neutrophils; mimics septic shock; causes release of ODFRs.</td>
<td>Neutrophils (proliferation, prolongs survival, enhances antibody dependent cytotoxicity and superoxide anion production); chemotactic for granulocytes and monocytes.</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>T-cells B-cells Macrophages Endothelial cells Fibroblasts</td>
<td>Proliferation, maturation and function of haematopoietic cells; causes release of ODFRs.</td>
<td>Neutrophils (proliferation, differentiation, prolongs survival, increases superoxide, leukotriene, PAF, arachidonic acid release, enhances phagocytic activity, inhibits IL-8 production and neutrophil migration); monocytes (proliferation, differentiation, induces IL-1, IL-8 and TNF release); chemotaxis; formation of adhesion molecules; angiogenesis; haematoipoiesis.</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Platelets Fibroblasts Monocytes</td>
<td>Stimulatory or inhibitory effects on proliferation and differentiation of many cell types depending on type of cell, growth conditions, cell differentiation state, and presence of other growth factors; modulates cellular and humoral immune responses; suppresses chemokine-mediated NO release.</td>
<td>Lymphocytes (suppresses B and T-cell proliferation, inhibits NK activity, inhibits IgG and IgM secretion, upregulates B-cell IgA secretion); macrophages (induces secretion of growth factors); chemotactic for macrophages.</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>Macrophages Endothelial cells Neutrophils Fibroblasts</td>
<td>Blocks the biological activity of IL-1 by competing for the IL-1 receptor.</td>
<td>Macrophages (enhances IL-1, IL-2 and TNF production); intracellular messenger in neutrophils; causes release of lysosomal enzymes; chemotactic for neutrophils; formation of adhesion molecules.</td>
</tr>
<tr>
<td>PAF</td>
<td>Macrophages Endothelial cells Neutrophils</td>
<td>Activates and aggregates platelets; mimics endothelial alterations of septic shock; induces release of ODFRs.</td>
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naturally occurring immunomodulators or their receptors [10]. Finally, the timing of any potential anti-cytokine therapy is clearly crucial [24]. Strategies designed to predict the activation of specific components of the inflammatory response may thus be useful. It is also possible that specific cellular targeting of such therapy may be more beneficial than global inhibition. Preliminary animal studies suggest that the therapeutic use of anti-inflammatory cytokines such as IL-10 and IL-13 may be beneficial in sepsis although as yet there have been no confirmatory clinical studies [6, 13].

References


4. Dripps DJ, Brandhuber BJ, Thompson RC, Eisenberg SP. Interleukin 1 (IL-1) receptor antagonist binds to the 80kDa IL-1 receptor but does not initiate IL-1 signal transduction. Journal of Biological Chemistry 1991; 266: 10331–10336.


