Cytokines in anaesthesia

P. SHEERAN AND G. M. HALL

Cytokines are a heterogeneous group of proteins, variously termed lymphokines, monokines, interleukines and interferons, which act on cell surface receptors to regulate and modify cell growth, maturation and repair. In addition to their longer-term effects on cell growth and differentiation, cell-mediated host-defence mechanisms and chronic diseases such as rheumatoid arthritis, cytokines also mediate several acute effects such as the inflammatory response. Cytokines are produced from activated leucocytes, in particular monocytes, and also from activated fibroblasts and endothelial cells. Activation of these cells is one of the earliest cellular responses to tissue injury and is associated with release of a variety of inflammatory mediators, including cytokines, arachidonic acid metabolites, complement split products, lysosomal enzymes and oxygen-free radicals. Cytokines derived from mononuclear phagocytes mediate non-specific immune responses and some parts of the metabolic response to injury. They have local and systemic effects, both mediated by activation of specific receptors. The other principal stimulus to the metabolic response to injury is afferent neuronal input from the injured or operative site.

This review is confined to activation of the cytokine network and the acute phase response found in surgery and trauma. Chronic stimulation of cytokine secretion, for example in sepsis, has been discussed recently.

Discovery of cytokines

Early studies of cytokines from 1950 to 1970 involved the description of numerous protein factors produced by different cells that mediated particular functions in vitro. Evidence implicated thymus-derived T lymphocytes as the cells which interacted specifically with antigen to release these protein factors, which were termed lymphokines to show their origin and function. Simple cell culture supernatants influenced the behaviour of target cells in many ways, indicating that cellular immune functions were regulated by soluble factors. It was at this time that antiviral interferons, fever-producing pyrogens and macrophage-activating factor were discovered.

The second phase of cytokine research involved the purification and characterization of many individual cytokines. It was realized that diverse effects were often mediated by the same peptide, and that cytokines were synthesized principally by leucocytes to act primarily on other leucocytes and thus were termed interleukins. However, preparations of cytokines at this time were often impure and many of the cytokine antibodies available were not specific. This hampered identification of, and distinction between, various cytokines.

In the past decade, molecular cloning and the production of highly specific, neutralizing antibodies, have resulted in the precise identification of the structure and properties of individual cytokines. All cytokines interact with specific receptors and it is the elucidation of the structure of these receptors and the signalling pathways used that are beginning to provide mechanisms and explanations for the often bewildering and diverse biological effects of this group of molecules.

Characteristics of cytokines (modified from Molloy, Mannick and Rodrick and Abbas, Lichtman and Pober)

Cytokines have the following properties:
1. They are low molecular weight (< 80 kDa) secreted proteins, often glycosylated.
2. They are involved in immunity and inflammation where they regulate the amplitude and duration of response. Cytokine secretion is a brief, self-limiting event.
3. Cytokines are extremely potent, generally acting at picomolar concentrations.
4. Cytokines, in common with other polypeptide hormones, initiate their action by binding to specific receptors on the surface of the target cell. Cytokines tend to be paracrine (act on a nearby cell) or autocrine (act on the same cell), rather than endocrine (secreted into the circulation to act on a distant cell).
5. Activation of surface receptors leads ultimately to a change in the pattern of cellular RNA and protein synthesis, and to altered cell behaviour.

Key words
Polypeptides, cytokines. Immune response, cytokines.

(Br. J. Anaesth. 1997; 78: 201–219)
(6) Cytokines act on many different cell types—pleiotropism.
(7) Cytokines often influence the synthesis and action of other cytokines.
(8) Cytokines often have multiple effects on the same cell.
(9) Cytokines act as regulators of cell division for many target cells, that is as growth factors.
(10) The response of a cell to a given cytokine is dependent on the local concentration of the cytokine, cell type and other cell regulators to which it is concomitantly exposed.

Most cytokines are synthesized as they are required and are not stored. However, some cytokines are presynthesized and stored in cytoplasmic granules. For example transforming growth factor-beta 1 (TGF-β1) is stored in alpha granules of platelets and released when stimulated by thrombin.9 Other instances of cytokines stored in this way include tumour necrosis factor-α (TNF-α) in some mast cells,56 granulocyte/macrophage colony stimulating factor (GM-CSF), platelet factor 4 (PF-4) and platelet derived growth factor (PDGF).69 Presynthesized cytokines can also be stored as membrane proteins, for example TNF-α, interleukin 1β (IL-1β), endothelial growth factor (EGF) and transforming growth factor α (TGF-α)78 or form complexes with cell surface binding proteins or extracellular matrix, for example transforming growth factor β (TGF-β), macrophage inflammatory protein 1β (MIP-1β) and interleukin-8 (IL-8).101 139 This serves as an immediate source of cytokine when the tissue matrix is broken down during injury and repair.

Role of cytokines in the immune response

The immune response is divided into the non-specific (or innate) and specific (or acquired) immune response. Non-specific defence includes physical barriers, several phagocytic cells and various blood-borne molecules. This defence mechanism is present before exposure to foreign macromolecules. It is not enhanced by exposure and it does not discriminate between foreign substances.

In contrast, the specific immune response is induced by exposure to foreign macromolecules. It is specific for each macromolecule and increases in magnitude with each successive exposure. In essence, the specific immune response serves to enhance the non-specific immune response.

NON-SPECIFIC IMMUNITY

When a foreign substance enters the body, the initial attempts to eliminate it come from an inflammatory response led by neutrophils and macrophages. In engulfing the foreign molecules, some of these cells extrude foreign antigen onto the cell surface, so becoming antigen presenting cells (APC). They are involved in the initial stages of specific immunity and also synthesize and release cytokines. Other active products of these cells are proteolytic enzymes and reactive oxygen species.

In conjunction with other cells of the immune system, such as natural killer (NK) cells and T helper lymphocytes (see below), macrophage activation is critically dependent on cytokine production. The complement system also plays a supportive role in the non-specific immune response. These proteins are synthesized in the liver and, when activated, augment phagocytosis and can cause direct cytolysis.

Other cells involved in this non-specific first line of defence are NK cells and lymphokine-activated cells (LAK cells). NK cells are large granular lymphocytes that destroy cells by direct contact, in a non-major histocompatibility complex-dependent manner. They can be regarded as cytotoxic lymphocytes (CTL) or CD8+ lymphocytes, without a T cell receptor for antigen, that is they have not resulted from overt antigenic stimulation. These cells were previously termed null cells, as they did not express markers for either T or B cells. While much is still not known about this group of lymphocytes, they kill their target cells by mechanisms similar to the CTL.

The major histocompatibility complex (MHC) is a region of polymorphic genes expressed on the short arm of chromosome 6, whose products (termed MHC-encoded proteins or MHC antigens) are expressed on the surfaces of a variety of cells.87 97 Thus the NK cell response, by acting in a non-MHC-dependent manner, is not antigen-dependent. NK cells also release TNF and interferons. In response to IL-2 and IL-12, NK cells are a potent source of interferon-γ (IFN-γ); in T cell deficient mice, IFN-γ from NK cells activates macrophages to provide a cellular response. LAK cells are formed when NK cells are exposed to high concentrations of IL-2 or interferon-α (IFN-α). They attack a broader range of target cells than NK cells, again in a non-specific manner. NK cells act in the initial response to a foreign antigen, attempting to eradicate the infected cell before the appearance of specific cytotoxic lymphocytes. If the stimulus is large enough, as in graft vs host reactions, NK cells differentiate into LAK cells, which, in common with other activated cells release cytokines to maintain the response.

SPECIFIC IMMUNITY

Specific immune responses occur after exposure to antigens, through a chain of events that results in highly specific antibodies (humoral immunity) or production of a specific set of lymphocytes (cell-mediated immunity).

The antigen receptors of B lymphocytes (bursa or bone-marrow derived lymphocytes) are membrane-bound forms of antibodies. Antigen binds to these membrane-bound antibody molecules to initiate the sequence of B cell activation, which culminates in the production of effector cells that actively secrete antibody molecules (humoral immunity).

T lymphocytes play a central role in specific immune responses to protein antigens. T lymphocytes can recognize and respond to foreign antigen only when it is presented in a complex with a self MHC molecule, on the surface of an appropriate APC cell. There are two different types of MHC molecule, class I and class II. Any given T lymphocyte can only recognize foreign antigen
Two types of T lymphocytes occur, T helper cells and cytolytic T cells. They can be differentiated from each other because they express different membrane proteins. These different membrane proteins can be recognized by antibodies, or more specifically a “cluster” of monoclonal antibodies. These membrane proteins are termed CD molecules or “cluster of differentiation” molecules. Most helper T cells express a surface protein termed CD4, and most cytolytic T lymphocytes express a different marker termed CD8. Apart from the invaluable role the CD antigens play in identifying, isolating and analysing the specificities of different lymphocyte subsets, CD antigens are also important in promoting cell–cell interactions and adhesion, and in transducing signals leading to lymphocyte activation.

Peptide fragments derived from extracellular protein (exogenous antigens), following phagocytosis, are expressed on the cell surface bound to class II MHC molecules and are recognized by CD4+ T cells (+ denotes activated cell). These CD4+ T cells are usually T helper lymphocytes. In contrast, peptides derived from proteins synthesized in the cell (endogenous antigens) from foreign genetic protein (viral), generally are expressed with class I MHC molecules. These are recognized by CD8+ T cells, which are usually cytolytic T lymphocytes. Therefore, whether an antigen is endogenous or exogenous determines the type of specific immune response.

There is a subset of T cells that may also inhibit immune responses, termed suppressor T cells. A major problem in the study of this group of lymphocytes has been inability to purify these cells in adequate numbers to analyse their response patterns and functions. Consequently, even basic questions remain unresolved. It seems the inhibitory effects of these suppressor lymphocytes may be mediated by suppressor proteins which are soluble receptors belonging to this lymphocyte subset, hence their antigenic specificity. What is clear is that some antigens can stimulate a group of lymphocytes whose principal role is one of down-regulation of major immune responses.

The T helper lymphocyte (Th cell) responds to the antigen on the surface of the APC. When activated, Th lymphocytes produce cytokines, which increase the number of cytokine receptors on cell membranes, resulting in further development of these T lymphocytes. Cytokines control the subsequent direction and development of T helper subsets, namely Th1 subset and Th2 subset, with each subset secreting discrete cytokine profiles. Thus bacterial stimuli activate cells of the non-specific immune system to produce IFN-γ, IL-2 and IL-12 and to drive Th1 development and cell-mediated immunity. Conversely, production of IL-4 early in the response favours Th2 development and an allergic/humoral immunity response.

Development of the appropriate Th subset is important because some pathogens are controlled most effectively by either a predominantly cell-mediated (Th1) or an allergic or antibody-mediated humoral immunity (Th2) type response. Maturation of the T helper subsets is cytokine dependent, Th1 is dependent on IFN-γ production, which is induced by IL-12, and predominantly produces further IFN-γ and IL-2. Th2 lymphocyte development is dependent on early IL-4 production and, on activation, produces IL-4, IL-5, IL-10 and IL-13, and little or no IFN-γ.

The concept of Th1 cytokines (IFN-γ, IL-2) stimulating Th1 lymphocyte development and suppressing Th2 lymphocyte activity, and Th2 cytokines (IL-4, IL-10, IL-13) stimulating Th2 development and IgE production and suppressing Th1 lymphocyte and IFN-γ activity may be one mechanism for “suppressor T cell” activity. Transforming growth factor-β (TGF-β), an inhibitory cytokine, is a powerful inhibitor of T and B cell proliferation, and any cell that produces excess amounts of this cytokine can play a role in suppressor T cell activity.

Thus cytokines play a major role in promoting or limiting Th1 or Th2 development, and also in initiating specific immune responses to various pathogens.

**CELL ADHESION MOLECULES (CAM)**

Together with the cytokines, CAM function as the molecular mechanism by which cells of the immune system communicate with each other, and appear on the surfaces of neutrophils and endothelial cells very early in an inflammatory response. Cell adhesion molecules are cell surface proteins involved in the binding of cells either to each other, to endothelium or to the extracellular matrix. They attract infiltrating phagocytic cells to the site of injury, in conjunction with release of chemotaxant agents (e.g. IL-8 and monocyte chemoattractant protein-1, MCP-1). Cell adhesion molecules primarily determine the migration route of cells, although they have other functions. Increased values of CAM may represent an earlier indicator of an activated immune response than an increase in cytokine secretion. The main groups of cell adhesion molecules are the selectins and immunoglobulin superfamily (IGSF). Selectins such as endothelial leucocyte adhesion molecule-1 (ELAM-1) act in the initial phases of cell adhesion to capture leucocytes from the circulation onto the vascular endothelium. This is only a transient interaction unless other adhesion pathways are activated.

IGSF adhesins include intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These are ligands for β1 and β2 integrins. This combination of ligand–integrin pairs is involved in establishing firm adhesion after the initial selectin-mediated adhesion. These pairs are also involved in the transfer of cells across endothelium to sites of inflammation.

**Classification of cytokines**

The principal biological actions of a particular cytokine fall into four broad categories, although many cytokines may function in more than one of these categories:
(A) MEDIATORS OF NATURAL IMMUNITY

This group includes those cytokines that protect against viral infection and those responsible for the protective inflammatory response against bacteria, such as TNF-α, IL-1, IL-6 (minor role) and the chemokines (cytokines whose principal effect is one of chemotaxis of leucocytes). Also included in this group are IL-5 (responsible for eosinophil chemotaxis) and IL-8 (responsible for neutrophil chemotaxis). Chemokines can be regarded as a distinct class of cytokine, in their own right.

(B) REGULATION OF LYMPHOCYTE ACTIVATION, GROWTH AND DIFFERENTIATION

Some cytokines are involved in the development of specific subsets of lymphocytes. The subset determines the type of immune response, namely IL-2 preferentially favours Th1 lymphocyte development and a cell-mediated immune response; IL-4 favours Th2 lymphocyte development and an allergic response (IgE production, mast cell development and adhesion molecule production responsible for eosinophil and basophil chemotaxis). TGF-β is an “anti-cytokine”, in that it largely counteracts the effects of the proinflammatory cytokines. It is included in this group of cytokines because it antagonizes many lymphocyte responses such as CTL maturation and activation of macrophages.

These cytokines are secreted in response to antigen recognition by lymphocytes, mediating the activation phases of specific immune responses. Most of the cytokines are produced by T cells, especially antigen-specific CD4+ T lymphocytes. Other examples include IL-1, IL-6, IL-12, IL-7, IL-9 and TNF.

(C) REGULATORS OF IMMUNE-MEDIATED INFLAMMATION

These cytokines activate non-specific inflammatory cells in response to specific antigen recognition by T lymphocytes. IFN-γ activates macrophages to phagocytose microbes and, with other cytokines regarded as macrophage activating factors (MAF), can also kill tumour cells. IFN-γ is the principal MAF, others include GM-CSF, and to a lesser extent IL-1 and TNF. IFN-γ also stimulates the cytolytic activity of NK cells, activates vascular endothelium and promotes Th1 development. Other cytokines included in this group are the “pro-inflammatory” cytokines IL-1 and TNF and the “anti-inflammatory” cytokines TGF-β, IL-4 and IL-10.

(D) STIMULATORS OF IMMATURE LEUCOCYTE GROWTH AND DIFFERENTIATION

This group includes cytokines already classified in some of the above groups; IL1, IL-3, IL-5 and IL-6. Immune and inflammatory reactions, which consume leucocytes, also elicit production of new leucocytes to replace inflammatory cells. These cytokines are collectively termed colony-stimulating factors (CSF), and specifically include granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF) and granulocyte/macrophage colony-stimulating factor (GM-CSF).

Cytokines relevant to anaesthesia

Cytokines play a central role in the acute inflammatory response initiated by trauma or infection. IL-1, TNF-α and IL-6 have local and systemic effects, which attempt to limit injury and the spread of infection, and provide a suitable environment for tissue healing and repair. Local effects include migration of neutrophils, lymphocytes and monocytes into inflamed areas as a result of increased permeability of the endothelium, adhesion molecules and chemotactic cytokines (chemokines) such as IL-8. Systemic changes include neutrophilia, fever production, adrenocorticotropic hormone release, a decline in circulating zinc and iron, and synthesis of acute phase proteins by the liver. These are all components of the acute phase response, a homeostatic response to major physiological insults such as trauma, infection or surgery.

There are numerous stimuli inducing release of TNF-α and IL-1; the most potent stimulus is the lipopolysaccharide or LPS component of endotoxin. Cytokines also induce further cytokine production, for example both IL-1 and TNF-α are potent inducers of IL-6, with IL-1 30 times more potent than TNF.

The physiology of each cytokine is outlined below.

TUMOUR NECROSIS FACTOR (TNF)

TNF is secreted as a pro-hormone and cleavage results in a 157 amino acid polypeptide. In common with all cytokines, interaction with a specific receptor is necessary for a biological response. Its potency is such that occupancy of as little as 5% of its receptors produces a biochemical response. It exists as two distinct polypeptides, TNF-α and TNF-β, which are antigenically different, yet bind to the same receptors and produce similar, but not identical effects. It shares a central role with IL-1 in initiating the cascade of inflammatory mediators, cytokines, complement, and activation of leucocytes, lymphocytes and macrophages that make up the immune response. Overproduction of TNF production can be disastrous to the host and is seen in such pathological conditions as cachexia, autoimmune disorders and meningococcal septicunia.

The two TNF proteins produce a diversity of effects because of (i) the ubiquity of their receptors, (ii) their ability to activate multiple signal transduction pathways and (iii) their ability to induce, or suppress, the expression of a vast number of genes coding for growth factors, cytokines, transcription factors, receptors, inflammatory mediators and acute phase proteins.

Just as there are two forms of TNF, α and β, so there are two forms of TNF receptor, TNFR-I and TNFR-II (see below). Both receptor types show equally high affinity binding for either TNF-α or
TNF-β. There is also present in serum soluble TNF binding proteins that are in essence TNF receptors not bound to cell membranes. These soluble receptors compete with the bound TNF receptors for TNF and therefore limit the activity of the cytokine.\textsuperscript{54,108}

INTERLEUKIN-1 (IL-1)

IL-1 was known by several names until amino acid sequencing revealed a closely related family of polypeptides. IL-1 exists in two forms, IL-1α and IL-1β, that are produced from two separate genes, yet activate the same receptors with equal affinity.

IL-1 has more inflammatory and immunoenhancing potential than TNF. It stimulates myelopoiesis directly, and also indirectly via several myelopoeitic growth factors, including GM-CSF. It is an endogenous pyrogen, increasing synthesis of prostaglandin \(E_2\) (PG\(E_2\)) in the anterior hypothalamus and is involved in the development of anorexia.\textsuperscript{86}

In common with TNF, the main stimulus for IL-1 release is LPS, endotoxin antigen. Indeed, both TNF and IL-1 share many functions and nearly all of the activities of TNF and IL-1 are enhanced when they are administered together.\textsuperscript{39} IL-1 and TNF have a wide variety of biological activities and play an important role in stimulating T and B cells, in addition to macrophages. TNF is chemotactic for polymorphonuclear leucocytes and activates respiratory burst and degranulation of these cells releasing oxygen-derived free radicals, whereas IL-1 does not. Both have antigenic activity, causing release of prostaglandins, IL-6 and IL-8 from monocytes, and stimulate endothelial cells to produce prostaglandins, IL-6 and tissue factor III, which may play a role in stimulating T and B cells, in addition to macrophages. TNF is chemotactic for polymorphonuclear leucocytes and activates respiratory burst and degranulation of these cells releasing oxygen-derived free radicals, whereas IL-1 does not.

IL-1 mediates its activities through receptors on the cell membrane, either IL-1R type I or IL-1R type II. They show 25% structural homology, and in general IL-1α binds to IL-1R type I and IL-1β binds to IL-1R type II.

It has been shown recently that all the biological effects of IL-1 can be attributed to the receptor IL-1R type II transducing a signal. The function of the IL-1R type II receptor is unknown, but it may serve as a precursor for the soluble receptor form, thus antagonizing and regulating the activity of IL-1.\textsuperscript{112,128}

INTERLEUKIN-6 (IL-6)

Together with TNF and IL-1, IL-6 is one of the mediators of the acute phase response. In addition to its role in the inflammatory response, it plays an important part in host defence, immune responses and haematopoiesis. Over-expression or chronic activation of IL-6 is implicated in diseases such as systemic lupus erythematosus and rheumatoid arthritis. IL-6 is expressed by several normal and transformed cell types and its production is up-regulated by numerous signals, including mitogenic and antigenic stimulation, lipopolysaccharide (LPS), IL-1, IL-2, TNF, interferon, platelet derived growth factor (PDGF) and viruses.

IL-6 exerts its activity through binding to a high affinity receptor complex comprising an 80-kDa receptor protein (IL-6R) and a 130-kDa signal transducing glycoprotein (gp-130).\textsuperscript{61} IL-6 binds to the receptor IL-6R with only low affinity. In the presence of gp-130, IL-6 binds to the receptor IL-6R with high affinity, resulting in signal transduction. In the absence of IL-6R, IL-6 does not bind to gp-130; however, in the absence of IL-6R and the presence of soluble IL-6 receptor (sIL-6R), high affinity binding with gp-130 occurs and signal transduction is triggered. This is in direct contrast to binding between TNF and the soluble TNFR, where the complex renders TNF inactive.

The regulation and physiological significance of this soluble IL-6R and its binding with IL-6 are poorly understood, but significantly increased serum concentrations of this protein have been found in some pathological states, such as multiple myeloma, adult T cell leukaemia and HIV infection. Soluble glycoprotein 130 has also been identified in serum and it is probable that this soluble protein regulates production of IL-6.\textsuperscript{35}

INTERLEUKIN-2 (IL-2)

IL-2 is the major autocrine and paracrine growth factor for T lymphocytes and the quantity of IL-2 synthesized determines the size of the T cell-dependent immune response. IL-2 also stimulates production of other T cell-derived cytokines, IFN-γ and TNF-α. IL-2 and IFN-γ encourage the development of the cytotoxic Th1 subset of T lymphocytes and are responsible for cell-mediated immunity. After injury PGE\(_2\) is produced by inhibitory monocytes and macrophages, stimulates growth and cytolytic function of NK cells, and is a growth factor for B cells.

The biological activities of IL-2 are mediated by binding to a multi-molecular cellular receptor, namely, IL-2Ra, IL-2Rβ and IL-2Rγ. Full details of signal transduction are not known but the IL-2Rγ receptor is common to several other cytokine receptors. As with other cytokine receptors, a soluble form of IL-2Ra exists. Its function is unclear, but it is an indication of T cell activation and may be able to predict the onset of transplant rejection.\textsuperscript{142}

IL-2 has shown promise as an anticancer drug because it stimulates LAK cells and tumour-infiltrating lymphocytes (TILs).\textsuperscript{117,148} High doses of IL-2 improved survival in a murine model of burns and sepsis.\textsuperscript{57} Furthermore, low doses of IL-2 combined with indomethacin improved survival and restored normal immune response in this murine model.\textsuperscript{65}
Antibodies to IL-2 and IL-2 receptors may have potential in the prevention of allograft rejection and suppression of auto-immune disease.

INTERLEUKIN-3 (IL-3)
IL-3 acts as a mast cell colony-stimulating factor (CSF) and a CSF on haematopoietic cells in bone marrow. It is regarded as a multilineage colony-stimulating factor and has similar actions to GM-CSF.

INTERLEUKIN-4 (IL-4)
IL-4 is involved, together with IL-5 and IL-13, in the differentiation of Th2 lymphocytes from immature Th (helper) lymphocytes. The main physiological function of this class of lymphocytes is the defence against helminthic infections and allergic responses. IL-4 is produced by Th2 lymphocytes and is the major cytokine involved in IgE production after B cell activation and proliferation. IL-4 stimulates expression of certain adhesion molecules (VCAM-1) on endothelial cells to aid rapid transfer of inflammatory cells, especially eosinophils to areas of inflammation. IL-4 inhibits activation of macrophages and the macrophage activating effects of IFN-γ, preventing production of Th1 cytokines, nitric oxide and prostaglandins. IL-10, another Th2 cytokine, shares these effects.

INTERLEUKIN-5 (IL-5)
IL-5 is a very potent chemotactic agent for eosinophils and acts as a stimulator of B cells. IL-4, IL-5, Th2 lymphocytes and eosinophils are found at sites of allergic reactions and circulating values may be increased in patients prone to allergies.

INTERLEUKIN-7 (IL-7)
IL-7 is an early growth factor of both B and T lymphocyte cell lines.

INTERLEUKIN 8 (IL-8)
This is a potent chemotactic agent for neutrophils which together with the inflammatory cytokines have been implicated in ischaemic/reperfusion injury. Pulmonary dysfunction after cardiopulmonary bypass is common and is thought to be caused by neutrophil-mediated endothelial damage and reperfusion injury. IL-8 release correlated significantly with the duration of cardiopulmonary bypass in paediatric patients.47

INTERLEUKIN 9 (IL-9)
IL-9 acts directly on T cell growth, and not merely as a cofactor for the major T cell growth factors such as IL-2, IL-4 and IL-7. It promotes mast cell growth in conjunction with IL-3.

INTERLEUKIN 10 (IL-10)
Most of the immunosuppressive effects of IL-10 result from inhibition of the activity of the antigen presenting cell (APC).16 IL-10 profoundly suppresses activation of macrophages, inhibiting their ability to secrete cytokines and to act as accessory cells for stimulation of T cell and NK cell function. It has been described as a cytokine synthesis inhibitory factor.

IL-10 down-regulates cell-mediated responses, suppressing the production of PGE2 and pro-inflammatory cytokines, IL-2 and IFN-γ.16,8 It also enhances release of soluble TNF receptor and inhibits the expression of surface receptor/adhesion molecule ICAM-1.146

The Epstein–Barr virus shares similar cellular activities with IL-10 and at least two of the herpes viruses harbour analogues of the IL-10 gene. This may be important in the host–virus relationship. Induction of IL-10 synthesis during infection may be an important strategy by which micro-organisms evade cell-mediated immune destruction and improve the chances of survival. IL-10 also stimulates B cell growth and differentiation.

INTERLEUKIN-11 (IL-11)
IL-11 has several effects on haematopoietic and non-haematopoietic cell populations. It has synergistic effects with IL-3 and IL-10 on megakaryocytes and pre-B lymphocytes.

INTERLEUKIN-12 (IL-12)
IL-12 is an important regulator of cell-mediated immune responses because it has multiple effects on T cells and NK cells and is a powerful inducer of IFN-γ production. IL-12 is an important initiator of Th1 cell development acting either directly or indirectly via induction of IFN-γ production. In the presence of IL-4, however, these effects are overcome, favouring the production of Th2 cells. IL-12 is also the most potent stimulator of growth and development of resting, or activated, NK cells and stimulates production of IFN-γ by these cells.

INTERLEUKIN-13 (IL-13)
This recently discovered cytokine has overlapping physiological properties with IL-4. It has 30% homology with IL-4 and has similar effects on macrophages, monocytes and B cells, but no effect on T cells.15

IL-13 can inhibit the production and expression of pro-inflammatory cytokines and, similar to IL-4 and IL-10, is regarded as a potent anti-inflammatory cytokine that suppresses cell-mediated immunity. IL-13 stimulates neutrophils to produce the IL-1 receptor antagonist, IL-1ra, which results in inhibition of the central inflammatory mediator IL-1.93

INTERLEUKIN 14 (IL-14)
IL-14 enhances the proliferation of activated B cells and stimulates immunoglobulin synthesis.5
INTERLEUKIN-15 (IL-15)

IL-15 shares many of the properties of IL-2, including stimulation of T lymphocytes. It is also responsible for in vitro generation of alloantigen-specific cytotoxic T cells and non-antigen specific lymphokine activated killer (LAK) cells. It is produced mostly by epithelial cells and monocytes, but is also found in a wide variety of cells.

The IL-15 receptor has the IL-2 receptor beta and gamma chains in its structure, but not the alpha chain.55

INTERLEUKIN-16 (IL-16)

IL-16 has no homology with other known cytokines and was previously known as lymphocyte chemotactrant factor (LCF). It is secreted from CD8+ cells and induces a migratory response in cells expressing the CD4 molecule, namely lymphocytes, monocytes and eosinophils. It may be that IL-16 has the ability to suppress HIV replication in CD8+-depleted monocytes.

PLATELET ACTIVATING FACTOR (PAF)

PAF is a family of compounds structurally related to acylglycerol-ether-phosphorylcholine, a membrane phospholipid. It is synthesized after activation of phospholipase A2 and released from platelets, basophils, neutrophils, monocytes and macrophages.46

PAF is also released from vascular endothelium by TNF, IL-1 and lipopolysaccharide (LPS) and has been closely linked to the profound shock seen in anaphylaxis and endotoxiaemia.129 A correlation between high concentrations of PAF and profound shock has been shown in patients with acute pancreatitis and severe infections (gram-negative septicemia, meningitis, malaria). In some animal models, PAF receptor antagonists offer partial protection.60 72 127

INTERLEUKIN-17 (IL-17)

IL-17 is a protein whose receptors are expressed by a wide variety of cells. It stimulates T cells and acts in a pro-inflammatory manner by activating the transcription protein NF kappa beta, resulting in increased expression of IL-6, IL-8 and ICAM-1 by fibroblasts.

INTERFERON-GAMMA (IFN-γ)

IFN-γ is produced by CD4+ and CD8+ T lymphocytes and NK cells and has potent immunoregulatory effects. In common with IFN-α and β, IFN-γ is an inhibitor of viral replication. IFN-γ influences the class of antibody produced by B cells, up-regulates class I and class II MHC antigens, and is an important initiator of the Th1 subset of lymphocytes and subsequent cell-mediated immunity. It increases the efficiency of macrophage-mediated killing of intracellular parasites. IFN-γ counteracts the effects of IL-4 and thus inhibits the activity of Th2 cells.90

INTERFERON-ALPHA (IFN-α), INTERFERON-BETA (IFN-β)

While there is only one gene for IFN-γ and IFN-β, there are 15 functional genes for IFN-α. IFN-α and IFN-β are produced by most cells in response to viral infection. They have potent antiviral activity, but at higher concentrations have antiproliferative activity against both normal and tumour cells. These activities have been exploited therapeutically.

TRANSFORMING GROWTH FACTOR ALPHA AND BETA (TGF-α AND β)

All cells have receptors for, and respond to, TGF-β. However, there is no structural or functional similarity between the two TGF molecules. Indeed, their roles may be opposed as TGF-α is a growth stimulator while TGF-β is a growth inhibitor (although it can be stimulatory for some cells of mesenchymal origin).

TGF-α is structurally similar to, and shares the same receptor as, epidermal growth factor (EGF), another important member of this large group of cytokine growth factors. EGF stimulates the basal epithelial cell layer of the skin and it can also stimulate bone resorption. The EGF receptor is a transmembrane tyrosine kinase receptor and is over-expressed in various human solid tumours and tumour-derived cell lines, suggesting it is involved in tumour progression.78 TGF-α has an important role in promoting cell proliferation in various tissues, for example the formation and resorption of bone, and wound healing.

TGF-β is found in the highest concentrations in human platelets and bone, but is produced by many cells. The actions of TGF-β are highly pleiotropic. As a cytokine, TGF-β is important because it antagonizes many lymphocyte responses; it inhibits T cell proliferation, maturation of CTL and macrophage activation. It acts on polymorphonuclear leucocytes and endothelium to counter the effects of pro-inflammatory cytokines. TGF-β can, therefore, be regarded as an anti-cytokine, as it is a negative regulator of immune responses. Leishmania braziliensis over-induces TGF-β production, thereby down-regulating the host’s immune response and increasing the virulence of this parasite.

Although TGF-β is essentially a growth inhibitor and may be important in preventing uncontrolled proliferation of normal cells in response to mitogenic growth factors, it plays a significant role in tissue repair and regeneration. It can accelerate wound healing by promoting production of the connective tissue components collagen and fibronectin, and is also involved in regulating bone remodelling.

Cytokine receptors

All known cytokine receptors are transmembrane proteins. The extracellular domain binds the
cytokine, thereby detecting the extracellular signal, and the intracellular domain either has enzymatic activity, binds other molecules or uses a second messenger system. Proteins also exist that are structurally similar to cytokine receptors or are able to occupy their binding sites, but are not attached to the cell membrane.

Cytokine receptors have been grouped into seven families determined either by the structure of the extracellular, presenting part of the cytokine receptor, the structure of the intracellular domain of the receptor or by the method of signal transduction. Many cytokines have differing combinations of extracellular domains that may qualify them for more than one family.

(1) RECEPTOR ONE, THE IMMUNOGLOBULIN RECEPTOR
This group usually displays one or more extracellular immunoglobulin domains. While it principally involves the receptors for IL-1α and β, it also includes some of the sequences of the IL-6R, macrophage colony-stimulating factor (M-CSF) and platelet derived growth factor (PDGF).

There are two distinct types of receptor (type I and type II). In general, type I binds IL-1α better than IL-1β, and type II receptor binds IL-1β more strongly than IL-1α. It has been suggested that the function of the membrane bound type II receptor is to serve as the precursor for a soluble IL-1 binding factor that can be shed under appropriate circumstances to antagonize and modulate IL-1 activity. A naturally occurring IL-1 binding protein has been described that corresponds to the soluble external portion of the type II receptor. This is the only naturally occurring cytokine receptor antagonist identified to date. It is structurally related to both IL-1α and IL-1β, and binds to the IL-1 receptors without causing signal transduction.

(2) RECEPTOR TWO
The second receptor to be classified represents the largest group, displaying various combinations of cytokine receptor domains, fibronectin type III (FNIII domain) and, in some cases, immunoglobulin domains.

These receptors bind cytokines that share a four α-helical strand structure, for example IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, G-CSF and GM-CSF. It was known previously as the haematopoietic receptor family.

(3) RECEPTOR THREE, INTERFERON RECEPTOR (IFNR)
This receptor type includes the type I and type II interferon receptors. One receptor type binds IFN-α and IFN-β and the other receptor binds IFN-γ. Most of the activities attributed to IFN-γ are mediated by IFN-γ proteins, which result from IFN-γ binding to its specific receptor.

(4) RECEPTOR FOUR, NERVE GROWTH FACTOR RECEPTOR (NGFR)
This fourth group of cytokine receptors, NGFR, includes the two TNF receptors that specifically bind TNF-α and β. The extracellular domains of these two receptors show similarities and can bind with equally high affinity either TNF-α or TNF-β. However, the intracellular domains of the two TNF receptors are unrelated, suggesting they transmit different signals. TNF-α and β use many signal transduction pathways which may explain how only a 5% occupancy of TNF receptors elicits a response.

Several groups have identified soluble TNF-binding proteins in human serum and urine. Two types of these soluble proteins have been identified; they represent truncated forms of the two TNF receptors. The soluble receptors have a concentration of approximately 1–2 ng ml⁻¹ in the serum and urine of healthy subjects. Concentrations vary from individual to individual but are stable over time in an individual.

Increased concentrations of these soluble receptors have been found in a variety of pathological states: endotoxaemia, meningococcaemia, cerebral malaria, systemic lupus erythematous, HIV infection and in the plasma and ascites of patients with infections and malignancies.

The soluble receptors are probably released from cells by proteolysis but the mechanisms are not well understood. There are reports of a correlation between increased circulating TNF values and soluble receptor concentrations. The precise physiological role of the soluble TNF receptor is unknown but these receptors can bind TNF in vitro. Thus shedding of soluble receptors scavenges TNF not bound to cell-surface receptors, limiting the effects of TNF and localizing the inflammatory response.

(5) RECEPTOR FIVE, G PROTEIN-COUPLED SEVEN TRANSMEMBRANE SPANNING FAMILY
The fifth type of cytokine receptor is a seven transmembrane α-helical structure displayed by the receptors of complement 5a, PAF, IL-8 and many related chemokines. This receptor is similar to the structure of the β-adrenergic receptor and rhodopsin. These receptors are coupled to GTP-binding proteins and activate kinases, phosphatases and ion channels.

Members of the chemokine family exhibit 20–50% homology in their predicted amino acid sequence and, depending on slight differences in the positions of these amino acids (a conserved four-cysteine motif), can be divided into an α- and β-subfamily.

(6) OTHER RECEPTOR TYPES
There is also a group of cytokine receptors that can be classified from their intracellular catalytic domain, tyrosine kinase. On activating this receptor, tyrosine kinase phosphorylates an intracellular transcription protein with further passage of the incoming signal. The seventh cytokine receptor family
type is the complement control protein superfamily.

(7) SOLUBLE RECEP'TORS AND SOLUBLE BINDING PROTEINS

The function of soluble receptors is not clear. In some instances, when bound to the ligand, they act as an agonist (sIL-6R) and in other cases as an antagonist (sTNFR). It is important to distinguish between soluble receptors (see above for TNF) and soluble binding proteins that have similar structures to cytokines and inhibit competitively the receptor (IL-1ra). Some of these soluble proteins are non-specific. Alpha-2-macroglobulin acts as a multi-functional binding protein, transporting cytokines to distant sites, but also removing them from the circulation.

In summary, soluble receptors and the soluble binding proteins can function as agonists, antagonists or simply as transport proteins, carrying cytokines to a distant target cell.

Signal transduction pathways

Signal transduction pathways are activated when a cytokine binds to its membrane-bound receptor. There are several different pathways for signal transduction and most involve protein phosphorylation. Some cytokine receptors have intrinsic enzyme activity (tyrosine kinase activity). Receptors with no intrinsic activity need a mechanism for linking with specific cytoplasmic proteins with enzymatic function. This is undertaken by either stimulatory or inhibitory G (GTP-binding) proteins. These molecules in turn regulate the activity of adenyl cyclase and the production of phosphokinase A, cGMP phosphodiesterase and possibly phospholipases.

SECOND MESSENGERS SYSTEMS

These systems amplify the initial cytokine signal. Cyclic-AMP–phosphokinase A is one of the best characterized second messenger systems. The activity of this complex can be regulated by phosphokinase C, which is stimulated by cytokines binding to their receptors.24 Other important second messengers include the phospholipases, enzymes that hydrolyse membrane phospholipids. Phospholipase A2 is the key enzyme in initiating arachidonic acid metabolism and prostaglandin and leukotriene production. Phospholipase C activity results in the production of inositol triphosphate (IP3) and diacylglycerol (DAG). These molecules are important second messengers and play crucial roles in the regulation of intracellular calcium (IP3) and in activating protein kinase C (DAG).25

Cytokines stimulating a cell may produce a rapid response or a response which is delayed for several hours or even days. At a cellular level, there is either up-regulation, down-regulation or a switching on or off of gene transcription which alters protein synthesis. One method of rapid transfer of the signal from the plasma membrane to the nucleus is by the use of inactive transcription proteins in the cytoplasm. These proteins are activated by phosphorylation and, when activated, migrate into the nucleus to produce the desired effect.

Signal transduction and gene transcription are currently areas of intense research as they offer possibilities for intervention and modulation of cytokine effects.

Cytokines and surgery

The cytokine response to surgery involves non-specific inflammation, specific immune responses, haematopoiesis and tissue repair.

THE ACUTE PHASE RESPONSE

The acute phase response to injury limits tissue damage, isolates and destroys the infective organism, and activates the repair processes that are necessary to return the host to normal function. Usually this response lasts only a few days. However, in cases of chronic stimulation of the inflammatory cascade, continuation of some aspects of the acute phase response may contribute to the underlying tissue damage that accompanies the disease (e.g. rheumatoid arthritis).

The acute phase response consists of:

(i) Alteration of the temperature set point in the hypothalamus and generation of a febrile response mediated through production of PGE2.

(ii) Change in plasma protein composition.

(iii) Increase in the number of granulocytes in peripheral blood, initially as a result of increased release from bone marrow stores and later from increased production.

(iv) IL-1 and IL-6 stimulate adrenocorticotropic hormone (ACTH) secretion and, consequently, production of cortisol. As corticosteroids inhibit cytokine gene expression, a negative feedback mechanism operates.

Acute phase proteins are synthesized in the liver, which increases in size during this response. These proteins are involved in non-specific/specific immunity as inflammatory mediators, scavengers and protease inhibitors, and in tissue repair. Acute phase proteins include: C reactive protein (CRP), which functions as a non-specific opsonin to augment phagocytosis of bacteria, alpha, macroglobulin and other anti-proteinases, and fibrinogen.

Over 30 proteins are involved in the acute phase response. Some can be used as, useful indicators of the presence and extent of an inflammatory process, for example C-reactive protein (CRP). The rate of increase in concentration of these proteins, and their rate of decline, varies from one acute protein to another. Changes in synthesis are caused by alterations in gene transcription in response to cytokine stimulation.

During the acute phase response plasma concentrations of albumin and transferrin, the iron transport protein, decrease. These are termed the negative acute phase reactants. Most of these changes can be attributed to alterations in the rate of synthesis of these plasma proteins by hepatocytes, while some are caused by increased catabolism.
There are also marked changes in serum concentrations of trace elements. Copper values increase, associated with an increased ceruloplasmin concentration, whereas there is a transient decrease in serum zinc and a persistent decrease in serum iron concentrations. This decline is caused initially by a decrease in serum values of the respective transport proteins (transferrin for iron, and albumin and alpha2 macroglobulin for zinc). This results from increased vascular permeability, which is aggravated after 6 h by reduction in binding of trace element to its protein.

Induction of the acute phase response

The macrophage or monocyte is usually the cell that starts the acute phase response at the site of tissue injury, by releasing a broad spectrum of mediators. These mediators, of which IL-1 and TNF-α are critical, initiate the next series of reactions. These early cytokines act both locally (on stroma cells—fibroblasts and endothelium) and distally, resulting in a secondary phase of cytokine release. Interleukin-6 is released in the second wave of cytokines and is a primary effector in the production of acute phase proteins and accumulation of inflammatory cells.

Circulating IL-6 values increase after all types of major surgery; increased concentrations can be detected as early as 30 min after skin incision and become significantly increased 2–4 h after surgery. The IL-6 response to surgery reflects the extent of tissue damage and is decreased when surgery is undertaken with laparoscopic techniques.

Patients with surgical complications have normal initial increases in circulating IL-6, but then develop a large secondary increase followed by a further increase in C-reactive protein.

IL-6, IL-1 and TNF-α have been shown in vitro to stimulate the release of ACTH from pituitary cells. Glucocorticoids inhibit IL-6 production in human monocytes but, by up-regulating production of the IL-6 receptor on hepatocytes, increase the acute phase protein response to IL-6.

IL-6, together with TNF-α and IL-1, can inhibit glutamine metabolism if incubated with human fibroblasts for 12 h. Impaired glutamine metabolism results in abnormal cell growth and consequent poor wound healing in critically ill patients. IL-6 activates platelets in vitro and enhances agonist-induced platelet aggregation via a mechanism involving cyclo-oxygenase eicosanoid metabolism. This effect may play only a small part in the overall hypercoagulable response to surgery but, in the coronary circulation, changes in platelet reactivity are temporally associated with the development of unstable angina.

It has been suggested that IL-6 may be a more sensitive indicator of the degree of myocardial damage after myocardial infarction than serial creatine kinase (CK) estimations, and an association was found between the size of the IL-6 response and cardiac morbidity. High concentrations of IL-6 impair cardiac function as a result of negative inotropic effects possibly consequent on impaired entry of calcium into myocardial cells. Other cytokines such as TNF-α, IL-2 and PAF also have negative inotropic effects. The effects of various anaesthetic techniques on the cytokine and acute phase responses to surgery are receiving increasing attention. Regional anaesthesia had little effect on IL-6 production after pelvic surgery, but it was shown that large doses of alfentanil transiently suppressed IL-6 secretion in a similar surgical model. The cortisol response to surgery is sufficient to down-regulate IL-6 production and large doses of glucocorticoids have been used to decrease the IL-6 and acute phase response to major abdominal surgery. Unfortunately this technique was associated with a high incidence of surgical problems such as wound dehiscence.

Cytokines and immunosuppression

Immunosuppression after major trauma results mainly from T cell dysfunction and is characterized by impaired synthesis of IL-2 and IFN-γ. The inability to produce adequate amounts of IL-2 causes incomplete T helper cell proliferation in response to antigen stimulation, and a lack of IFN-γ results in impaired ability of monocyte antigen presentation. These two cytokines are crucial to the development of the Th1 subset of lymphocytes that are responsible for cell-mediated immunity. This down-regulation of IL-2 and IFN-γ appears to be a direct consequence of PGE2 production in the acute phase response. Other immunosuppressants include TGF-β, IL-10 and the naturally occurring antagonists, sTNF-α and IL-1ra. TGF-β induces further production of PGE2 in monocytes and in some environments may produce more cell-associated TNF-α. It also suppresses IL-2- and IL-4-dependent Th development and down-regulates the T cell receptor for IL-1. McBride and colleagues have recently identified significant amounts of circulating IL-10 in patients after cardiopulmonary bypass.

In brief, the principal immunological deficit after trauma and major surgery is decreased cell-mediated immunity from an impaired NK cell response and Th lymphocyte development. This results in preferential Th2 development, probably as a result of suppression of Th1 development. Recent anaesthetic reports suggest there may be some benefit from infusing cytokines, such as interferon α and β, and IL-2, on NK cell activity.

Cytokines and wound healing

Cytokines have profound effects on various aspects of granulomatous tissue formation and re-epithelialization, critical problems in burns patients and patients with chronic ulcers. The cytokines primarily involved are TGF-β, PDGF, fibroblast growth factor (FGF), EGF, IL-6 and the proinflammatory cytokines TNF-α, IL-1α and β. The role of TGF-β in wound healing seems contradictory, as it is a potent growth inhibitor for many cell types.
However, TGF-β is usually stimulatory for cells of mesenchymal origin and inhibitory for cells of epidermal origin. There are numerous links between TGF-β and the macrophage, which plays a pivotal role in directing cellular responses required for wound healing. In addition to stimulating its own gene expression, TGF-β can stimulate other growth factors, such as FGF and PDGF. TGF-β is also chemotactic for monocytes and fibroblasts, encouraging their passage into the wound site, and has been shown to increase the strength of wound repair in experimental animals.

PDGF can influence all cells involved in soft tissue repair and is released at wound sites by platelet α granules. Further release of PDGF is continued by activated macrophages and fibroblasts. In conjunction with EGF, other peptides, such as insulin-like growth factors (IGF) help tissue repair. It has also been demonstrated that TNF-α and IL-6 promote healing, although over-production can have a deleterious effect. There is evidence that IL-6 values in wound fluid are higher than corresponding serum values, although this may reflect the extent of local tissue damage. Fahey and colleagues suggest that diabetes may obtund this IL-6 response, contributing to delayed wound closure.

In bypass vascular techniques, the main cause of vein graft failure in the early postoperative period is intimal hyperplasia which may be mediated by cytokine growth factors.

CYTOKINES AND THE EXTRACORPOREAL CIRCULATION

One of the most potent stimuli for leucocyte activation, and therefore cytokine production, is exposure of blood to artificial extracorporeal circuits. In clinical practice this concerns patients undergoing renal dialysis and cardiopulmonary bypass (CPB).

There is much conflicting data on cytokines and renal failure with some groups showing an increase in circulating values and other workers finding no change. The main stimulus to cytokine production is the dialysis procedure and not the underlying chronic renal failure. Pereira and Dinarello suggested that the variability reported may simply reflect the wide spectrum of cytokine-secreting ability in these patients, as similar variation has been noted in healthy volunteers. Other factors associated with renal failure such as poor nutrition and anaemia can also affect cytokine production. There is more inhibitory activity in plasma from haemodialysis patients than plasma from patients with renal failure and normal subjects. For example, a threefold increase in IL-1ra and soluble TNF-receptor values was found in patients receiving chronic haemodialysis.

Cardiac surgery compounds the inflammatory response triggered by the extracorporeal circulation, first by the severity of the surgery and second by the greater damage to the cells in the CPB circuit resulting from high flows and passage through the oxygenator. The cytokine response to cardiac surgery has received considerable attention and was reviewed recently. In brief, the main inflammatory cytokines are IL-6 and IL-8, with only minor increases in IL-1 and TNF. The anti-inflammatory cytokine IL-10 responds rapidly to CPB, and other cytokine antagonists such as IL-1ra increase significantly. Cardiac surgery has a marked suppressant effect on cell-mediated immunity.

Cardiopulmonary bypass results in activation of polymorphonuclear neutrophils and their adhesion to, and chemotaxis through, vascular endothelium. This may be because of expression of CD 11b and CD18 on the neutrophil and endothelial cells and expression of the adhesion molecule L-selectin. IL-8 is a neutrophil chemoattractant that induces the cell adhesion molecules CD11 and CD18. Reperfusion injury is a major cause of morbidity after CPB, particularly in myocardium and lung tissue. It seems that the neutrophil, through generation of oxygen-free radicals, upsets the balance between radical-generating and radical-scavenging systems, resulting in reperfusion injury. Detailed discussion of this area falls beyond this article, and is covered well in other reviews.

CYTOKINES AND COAGULATION

Cytokines do not directly participate in coagulation reactions, but they may accelerate, or decrease, the rate of specific pathways. Thus, TNF-α and IL-1 activate the procoagulating mechanisms in vivo, but do not alter the clotting time. The endothelium has both procoagulant and anticoagulant activities and, in the quiescent state, the latter predominates to favour blood fluidity. Cytokines can alter endothelial cell function; IL-1 increases endothelial procoagulant activity while decreasing expression of thrombomodulin. Fibrinolysis is also inhibited by IL-1, with decreased tissue plasminogen activator (tPA) and enhanced plasminogen activator inhibitor-1. TNF induces tissue factor, suppresses thrombomodulin, depresses endothelial fibrinolytic activity and increases the permeability of the endothelial monolayers.

Abnormalities of the coagulation system are a common finding in inflammatory, immune and neoplastic diseases. Cytokines, forming a key link between host response and the coagulation mechanism, modulate expression of cell surface cofactors, and factors and mediators released from endothelium and monocytes.

CYTOKINES AND THE VASCULAR ENDOTHELIUM

Features of diseases that involve large blood vessels include dysfunction of the endothelial lining, further stimulating mediators of the inflammatory response, weakening of the artery wall and narrowing of the arterial lumen. Cytokines, and other growth factors, are responsible for the expression of cell adhesion molecules and cell migration into localized areas of vessel injury. Vascular endothelial growth factor (VEGF) stimulates endothelial cells but not smooth muscle production, while platelet derived growth factor (PDGF) has no effect on endothelial cells, but stimulates smooth muscle cell...
production. Fibroblast growth factor stimulates both types of cell proliferation. These growth factors, sequestered in low concentrations in normal vessels, are released after injury; their action is localized by selected expression on cells and removal by the circulation.\textsuperscript{110}

Cell adhesion molecules, notably vascular cell adhesion molecule (VCAM), encourage migration of monocytes and lymphocytes. TNF-α, IL-1 and IFN-α alter vascular permeability by interfering with the endothelial layer.\textsuperscript{130} Changes in the contractile state of the arterial smooth muscle, in the absence of endothelium, have been shown with at least four cytokines (EGF, PDGF, TGF-β, IL-1). In addition, the IL-1 induced generation of nitric oxide may explain the hypotension seen after administration of this cytokine.\textsuperscript{13}

### Cytokines and the nervous system

#### CENTRAL NERVOUS SYSTEM

It had been thought that cytokines, usually associated with the peripheral immune response, would only selectively cross the intact blood–brain barrier. Circulating cytokines are able to stimulate those parts of the CNS outside the blood–brain barrier (circumventricular organs), resulting in a febrile response and release of corticotrophin releasing factor (CRF).\textsuperscript{120} Overproduction of some cytokines, such as TNF-α in bacterial meningitis, may lead to tissue injury and breakdown of the blood–brain barrier.\textsuperscript{125} It has been demonstrated that while the pro-inflammatory cytokines may not be responsible for this breakdown in integrity of the blood–brain barrier, they are able to cross the disrupted barrier. Saturable transport systems exist for IL-1 and TNF-α, but not for IL-2.

Leucocytes are able to cross the blood–brain barrier as part of an inflammatory response and secrete cytokines. Cellular elements of the CNS such as neurones, glial and endothelial cells are also able to produce cytokines. Gliial cells (the microglia is the brain’s macrophage) can be activated by IL-1 and TNF-α, produced by T lymphocytes that have migrated into the CNS. These gliial cells, after activation, can express either class I or class II types of MHC antigen to either subset of T lymphocyte.

Not only do cytokines have central effects resulting from systemic disease, they also have important roles in acute and chronic neurological diseases. IL-1, at low concentrations, stimulates production of nerve growth factor (NGF) and fibroblast growth factor (FGF), and is neuroprotective. However, in high concentrations, it causes massive exacerbation of the primary insult. IL-1ra markedly limits the extent of damage induced by focal ischaemia or activation of N-methyl-D-aspartate (NMDA) receptors in such diverse conditions as stroke, meningitis, excitotoxic damage and heat stroke.\textsuperscript{118} Cytokines influence the production and effects of neurotransmitters and are also involved in tissue repair as IL-1 and IL-6 enhance the expression of NGF, and TNF-α initiates glosis. Cytokines also have behavioural effects, altering sleep patterns and the social response to sickness.

### CYTOKINES AND PAIN

Inflammation is a painful process and recent reviews have emphasized a role for the primary mediators of the cytokine cascade, TNF-α and IL-1β in hyperalgesia.\textsuperscript{45,51} Hyperalgesia induced by IL-1β depends partly on induction of bradykinin-1 receptors. It is not surprising, therefore, that pharmacological block of bradykinin and cytokine activity is an area of active pain research.\textsuperscript{111} Bicyclic imidazoles, such as tenidap, are under intensive investigation as they have been shown to modify the progress of rheumatoid arthritis by partly interfering with cytokine production.\textsuperscript{73}

However, recently attention has been centred on nerve growth factor (NGF). NGF regulates nociception by a direct action on primary afferent fibres, with increased amounts present in inflammation, sunburn and rheumatoid arthritis, and decreased amounts in leprosy, neuropathies and in ageing. NGF modulates the production of substance P and is a key molecule in the understanding of the phenomena of allodynia and hyperalgesia.\textsuperscript{8} An anti-NGF preparation has been developed which will help clarify the biological activity of NGF and may increase our understanding of chronic pain.

### Cytokines and pregnancy

There is increasing evidence that cytokines, in addition to the hypothalamic, pituitary and gonadal hormones, play an important part in reproductive function.\textsuperscript{119} Several cytokines are involved in pre- and peri-implantation developments, in maternal–fetal interactions and in the initiation of parturition. Enhanced cytokine production may be partly responsible for problems developing in the antenatal period, such as preterm delivery\textsuperscript{75} and pregnancy-induced hypertension.\textsuperscript{76}

The development of the pre-implantation embryo is more successful and rapid in the uterus than in vitro. This suggests that, while the process is autonomous, it is stimulated in the uterine environment. It is likely that epidermal growth factor (EGF), insulin-like growth factors (IGF) and leukaemia inhibitory factor (LIF) are involved in embryo development.\textsuperscript{119}

The paternal component of the fetal genome represents a challenge to the maternal immune system. Therefore, modifications to the maternal immune response, both peri-implantation and as the pregnancy develops, occur to avoid rejection of, what is in effect, a partial allograft. Cytokines are important in communication between the developing blastocyst, maternal uterine cells and infiltrating leucocytes before implantation and as the placenta develops.\textsuperscript{113}

Cell-mediated immunity is decreased in pregnancy.\textsuperscript{49} A relative increase in the Th2 lymphocyte population is found, characterized by enhanced production of IL-4 and IL-10 with increased immunoglobulin production and decreased Th1
activity. IL-2 and IFN-γ (Th1 cytokines) facilitate rejection of the developing allograft and represent one example of how upsetting the balance between Th1 and Th2 cytokines has implications for the clinical course of several autoimmune diseases associated with pregnancy.49,145 In pre-eclampsia or pregnancy-induced hypertension, increased cell-mediated immunity seems to be partly responsible for an underlying chronic rejection pattern.76

Magnesium is used increasingly as an anti-epileptic, sedative and hypotensive agent in the treatment of severe pre-eclampsia. This element is a non-specific, down-regulator of cytokine activity, probably by antagonizing increased intracellular calcium that is an essential signal after stimulation by cytokines.144 Thus magnesium may work, at least in part, by inhibiting the effects of excessive cytokine activity.

Cytokines and hypersensitivity

The immune response to allergens is virtually identical to the response to parasites, with large amounts of immunoglobulin E (IgE) produced by B lymphocytes. These B lymphocytes are stimulated by Th cells which recognize antigen displayed on the surface of the antigen presenting cell (APC). These APC stimulate the production of IL-4 which causes the B cells to produce IgE and encourages further differentiation of Th helper cells into Th2 lymphocytes with production of the “allergic” cytokines IL-4, IL-5 and IL-13. In contrast, IFN-γ inhibits this pathway of Th2 cell development and encourages development of Th1 lymphocytes and their specific cytokines IL-2, IFN-γ and TNF-β.80,116

Atopic allergy is a genetically determined disorder, characterized by an increased ability of B lymphocytes to produce IgE antibodies to certain forms of ubiquitous allergen, present in low concentration. Essentially, the IgE produced attaches to the Fc fragments of IgE receptors on the surface of mast cells. When the mast cell is next exposed to that antigen, the antigen binds to its IgE receptors, resulting in mast cell, degranulation. This degranulation results in the release of vasoactive mediators, chemotactic factors and cytokines that are responsible for the allergic cascade. In addition, eosinophils are also involved, as they invariably accumulate at the sites of allergic inflammation, and the toxic products released contribute significantly to the resulting tissue damage.

Mast cells play not only a central role in immediate allergic responses, but also serve an immunoregulatory function, through production of IL-3, IL-4, IL-5, IL-6 and TNF, and chemokines such as IL-8 (neutrophil chemotaxis).27 Specific immunotherapy can change the cytokine profiles of the allergic Th2 cells and result in either increased Th1 cells or reduced synthesis of IL-4.128 IFN-γ stimulates Th1 lymphocyte development at the expense of IL-4 production and hence inhibits Th2 lymphocyte development and causes a decrease in circulating IgE values. In asthma, therapy with an anti-IL-5 compound has resulted in decreased airway responsiveness. IL-4 must be an attractive target for pharmacological intervention because of its role in IgE switching, development of Th2 lymphocytes and induction of eosinophilia.134 Glucocorticoids are the most potent drugs for controlling airway inflammation, but seem to have their principal effects on inhibiting Th2-type cytokine production (on IL-5 rather than IL-4)70 and bronchial eosinophilia.114

Anaphylactic reactions during anaesthesia occur more commonly in patients with an atopic history. It may be possible to predict those atopic patients at risk of a hypersensitivity response by assessing their “state of reactivity”, that is circulating concentrations of IL-4, IL-5, IL-13 and eosinophil count. Some studies have already attempted to correlate IL-4 with IgE values.79 TNF and platelet activating factor (PAF) are important mediators of anaphylaxis. The use of PAF antagonists to obtund the amplification of mediator release in shock has been studied in animal models, where even delayed receptor block had some benefit.80,127

Cytokines play a central role in IgE synthesis and in the further differentiation and expansion of the various cell lines involved in the allergic response. Atopy is genetically determined but, whether this is a result of increased expression of Th2-type cytokines, decreased expression of Th1-type cytokines or a combination of the two has not been fully determined. Targeting the Th2 lymphocytes and their cytokines seems a promising approach to reduce the chronic inflammatory response of allergy and asthma. In acute and massive mediator release, as occurs in anaphylaxis, specific cytokine receptor antagonism has improved outcome in animal models.

Cytokines and transplantation

Solid organ and bone marrow allografts represent unique forms of generalized activation of the immune system because of histoincompatibility. Many of the complications of these procedures, including rejection, graft vs host disease (GVHD), interstitial pneumonitis, vs host disease and microangiopathy can be related to inadequately regulated cytokine production.54,81

Patients in the early weeks after bone marrow transplantation are at particular risk of bacterial and fungal infection, the incidence and severity of which are related to the period of neutropaenia. Cytokine growth factors such as granulocyte colony stimulating factor (G-CSF) and granulocyte/macrophage colony stimulating factor (GM-CSF), stimulate myelopoiesis. If these factors are administered during this time, the severity and duration of neutropaenia and therefore the risk of infection, are reduced.

Cytokines play a central role in the rejection response to allograft organ transplantation.32 The rejection response can be divided into two phases, an afferent and efferent response. The afferent response leads to activation of the recipient’s immune system, as a result of recognition of foreign major histocompatibility antigens (class II type MHC or
FK506, a much more potent immunophilin binding and also improve rescue treatments. Newer agents give excellent primary immunosuppression and the effects. The immunophilin binding drugs, such as glucocorticoids, cyclophosphamide, azathioprine, and anti-T-cell antibodies have been used to reduce inflammation in autoimmune diseases and have been identified in the transplanted graft during rejection.32 IL-1, IL-2 and IFN-γ stimulate the development of Th1 type lymphocytes expressing CD4+ antigen that recognize class II MHC molecule. Occasionally, when mismatch occurs to class I MHC antigens only, CD8 + lymphocytes, the cytotoxic lymphocytes, can act as T helper cells. IFN-γ also up-regulates MHC antigen expression on graft tissue, resulting in increased vulnerability of the graft. IL-2 and IFN-γ play a central role in the rejection process. The presence of one of these cytokines within the graft may be a sign of early rejection.

The efferent arm of the response occurs as a later event and results in triggering of the humoral and cellular effector mechanisms of the immune response. Immune effector mechanisms involved include delayed-type hypersensitivity reactions, NK cells, LAK cells, antibody-dependent cell-mediated cytotoxicity and complement-dependent antibody-mediated cytotoxicity.

Immunosuppression has been the traditional form of cytokine down-regulation. Non-specific agents, such as glucocorticoids, cyclophosphamide, azathioprine and anti-T-cell antibodies have been used to reduce inflammation in autoimmune diseases and post-transplantation surgery. All of these agents are directed against the T cell compartment and have more general and non-selective immunosuppressive effects. The immunophilin binding drugs, such as cyclosporin and FK506, are more selective and the newer agents give excellent primary immunosuppression and also improve rescue treatments. FK506, a much more potent immunophilin binding drug than cyclosporin, is particularly promising.52 It inhibits the production of cytotoxic lymphocytes, specifically by blocking the synthesis of IL-2, IL-3, IL-4, GM-CSF, G-CSF, TNF and IFN-γ. As improved immunosuppression reduces the graft failure from acute rejection, chronic rejection becomes more obvious. Differentiation between acute and chronic rejection is based on time rather than the mechanisms involved.

Down-regulation of cytokines

Cytokine activity plays a fundamental role in the pathogenesis of many inflammatory diseases. Down-regulation of cytokines, or block of their effects, can occur at various sites. The principles of influencing cytokine production, or immunomodulation, will be discussed only in outline.

(i) Immunosuppression

(a) Glucocorticoids have a direct impact on cell proliferation and inhibit cytokine production.

(b) Azathioprine and cyclophosphamide inhibit cell division and disrupt DNA transcription and RNA translation resulting in decreased cytokine production.

(c) Anti-T-cell antibodies have a direct effect on T lymphocytes and therefore result in decreased T cell function. Naturally occurring autoantibodies against IL-1α, IFN-α, IFN-γ, TNF-α, nerve growth factor (NGF) and IL-6 have been isolated from normal individuals and patients with acute inflammatory disorders. The significance of these auto-antibodies is unknown. Synthesized antibodies (monoclonal antibodies—MAbs) have been developed against cytokines and cytokine receptors. This therapy has also been used with some success against T cell surface markers and leucocyte adhesion molecules.

(d) Immunophilin binding drugs—cyclosporin, FK506. These drugs inhibit immune responses more selectively by forming complexes with cytoplasmic transcription proteins that interfere with calcium-dependent signalling pathways.30 IL-2 production is inhibited and hence Th1 lymphocyte development blocked.

(ii) Blockade of signal transduction

When TNF binds to its receptor, it activates multiple signal pathways which may be manipulated by specific enzymatic inhibitors. For example, pentoxifylline inhibits TNF production in vitro. It is a xanthine derivative, and acts by inhibiting phosphodiesterase resulting in increased intracellular cAMP and decreased cellular mRNA concentrations of TNF. Another important agent is ATP–magnesium chloride (ATP–MgCl2). Magnesium deficiency increases circulating IL-1, IL-6 and TNF-α in rodents.14 Calcium channel antagonists may also change the cytokine response. For example, diltiazem enhanced production of cytokines and maintained immune function after haemorrhage.82

(iii) Cytokine receptors and receptor antagonists

Cytokine receptors can be targeted in several different ways:

(a) antibodies against receptor;

(b) soluble proteins binding competitively to membrane-bound receptor;

(c) development of amino acid substitution in cytokines.

The two most studied naturally occurring specific inhibitors of cytokines are IL-receptor antagonist and soluble TNF receptors. Biological responses to IL-1 can be induced by occupancy of as few as 5% of IL-1 receptors, and in vivo up to 100-fold excess of IL-1ra is required to inhibit 50% of IL-1 induced responses.40 This indicates the presence of a large number of IL-1 receptors on cells compared with the number that need to be occupied to trigger a biological response.40 As the pattern of production and regulation for IL-1 and IL-1ra is different, the exact relationship between the two cytokines remains to be established.8
Soluble TNF receptors represent the cleaved extracellular domains of the TNF receptors, type I and type II. Their functions are complex; they may serve as TNF inhibitors, TNF reservoirs or, in some situations, even augment TNF effects by stabilizing its tertiary structure and prolonging its action.2

(iv) Alpha-2-macroglobulin
This is a non-specific, multi-functional binding protein that serves as a proteinase inhibitor, a carrier protein and a cytokine modulator. Other examples of naturally occurring non-specific inhibitors of cytokines include lipoproteins and lipids.38

CYTOKINE DOWN-REGULATION OF IMMUNE FUNCTION
There are several cytokines, TGF-β, IL-10 and IL-4, whose normal function is to inhibit further cytokine development.

(i) Transforming growth factor B (TGF-β)
TGF-β promotes extracellular matrix formation and stimulates some mesenchymal cells, but also inhibits the growth of a variety of normal and transformed cells, including B cell maturation and release, NK cell activity and release of cytokines by T cells.

(ii) IL-10
Interleukin-10 is produced by a selected subset of Th2 cells, macrophages and B lymphocytes with the other Th2 cytokines, IL-4 and IL-5.48 IL-10 also inhibits presentation to Th1 cells, up-regulation of the MHC class II antigen and production of TNF-α, IL-1 α, IL-1 β, IL-6 and IL-8.38 However, IL-10 up-regulates B cell proliferation, differentiation and immunoglobulin synthesis. It suppresses the production of Th1 cytokines (IL-2, IL-3, IFN-γ, TNF-β and GM-CSF).

(iii) IL-4
IL-4 induces production of IL-10, a cytokine inhibitory factor, as detailed above. It also increases the production of CD8+ cytotoxic cells and decreases the number of CD4+ cells, NK cells, IL-2 activated killer cells and down-regulates IFN-γ.106

Summary
The scope of cytokine biology is fascinating with widespread applications anticipated in many areas of medical science. We have emphasized the fundamental roles that cytokines play in the control of normal immunological functions, defence mechanisms and normal cell growth, and how major surgery and trauma can affect these processes. It remains to be determined if morbidity and mortality may be improved after major illness and surgery by altering cytokine production and function.

References
26. Clemens MJ. How do cytokines work? 3.2.6 Diacylglycerols by stem cell factor IL-3 or IL-4. Regulation of mouse peritoneal mast cell secretory function by stem cell factor IL-3 or IL-4. Immunology Today 1992; 13: 331–336.
Cytokines in anaesthesia


75. Lockwood CJ. Recent advances in elucidating the pathogenesis of preterm delivery, the detection of patients at risk, and preventative therapies. Current Opinion in Obstetrics and Gynecology 1994; 6: 7–18.


143. Weglicki WB, Philips TM, Freedman AM, Cassidy MM, Dickens BF. Magnesium-deficiency elevates circulating...


