

Overview

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Cytokines are low molecular weight protein mediators involved in cell growth and differentiation, inflammation, immunity, and repair. Lymphocyte derived molecules were named *lymphokines* and products of monocytes *monokines*, but it is now known that there is considerable overlap between lymphokines and monokines—for example, molecules such as tumour necrosis factor α (TNF α) or interleukin 6 (IL-6) are made by both cell types—and these terms are thus of limited value.

The term *interleukin* means molecular messenger acting between leucocytes. Currently there are 13 molecules with a wide range of activities designated as interleukins 1–13. It is becoming clear that these molecules not only act on cells other than blood cells, but also are produced by cells other than blood cells such as fibroblasts or endothelial cells. The term cytokine also includes molecules such as the interferons or growth factors that have effects against viruses, stimulate growth of haematopoietic cells, and regulate cellular activation. A new family of low molecular weight (8–10 kDa) cytokines—the *chemokines*—has recently been described; among these are IL-8 and the monocyte chemoattractant protein (MCP-1) which are potent chemotactic factors. A summary of the cytokines is given in table 1.

Cytokines are chiefly involved in events in the local milieu, unlike hormones which are transported via the blood stream to their target organs throughout the whole body, and their major physiological role is exerted within a few cell diameters, although some such as IL-1 or TNF α do have systemic effects. Most of the cytokines are in the 10–25 kDa mass range. They are usually not produced constitutively and are effective in the picogram (pg) to nanogram (ng) range. When released their half life is short.

The effects of cytokines are mediated after binding to high affinity receptors on the cell surface. Receptors are usually present in low numbers (100–1000/cell), but exhibit high affinity. Cytokine receptors are subject to a variable degree of upregulation upon cell activation. For the IL-2 receptor β chain this phenomenon can be spectacular: 0–10 receptors on resting T cells up to 50 000 per activated T cell in three days. This upregulation is usually transient, lasting a few days in the absence of further stimulation. Upon interac-

tion with their ligand, cytokine receptors are typically internalised, taking the ligand with them. Receptors become re-expressed a few hours later. After cytokine exposure, cytokine receptor expression therefore varies considerably, diminishing initially as a result of its internalisation and then increasing because of the cytokine induced receptor upregulation.

This overview will concentrate on the effects of cytokines on target cells of most relevance to the lung and will illustrate some of their functional properties and the complexity of their interactions with various cell populations. The other three articles in this series will focus on the effects of cytokines on the processes of inflammation, injury, and repair.

Cytokines and endothelial cells

Endothelial cells are strategically located at the interface between circulating blood elements and tissues.¹ Cytokines are communication signals in the complex bidirectional interaction between leucocytes and endothelial cells. Endothelial cells represent a source of cytokines and, at the same time, a target for the action of these mediators.² Cytokines such as IL-1 or TNF α enhance the adherence process for leucocyte migration or modify membrane permeability. Interferon (IFN) γ activates the accessory cell function of

Table 1 Cytokines

Interleukins
IL-1 α , IL-1 β , IL-1ra, IL-2 to IL-13
Colony stimulating factors (CSF)
GM-CSF, G-CSF, M-CSF (CSF-1), EPO, LIF, SCF
Tumour necrosis factor
TNF α , TNF β (lymphotoxin)
Chemotactic factors
Chemokines α : IL-8, GRO/MGSA, PF4, NAP-2, huMIP-2a
Chemokines β : huMIP-1a, huMIP-1b, RANTES, I-303, MCP-1, MCP-2, MCP-3
Growth factors
EGF, TGF- α , aFGF, bFGF, PDGF A, PDGF B, ECGF, IGF-1, IGF-2, NGF β
EPO—erythropoietin; LIF—leukaemia inhibitory factor; SCF—stem cell factor; GRO/MGSA—growth related oncogene/melanoma growth stimulating activity; PF4—platelet factor 4; NAP-2—neutrophil activating protein; huMIP—human macrophage inflammatory protein; MCP—monocyte chemoattractant protein; EGF—epithelial growth factor; TGF—transforming growth factor; FGF—fibroblast growth factor; PDGF—platelet derived growth factor; ECGF—endothelial growth factor; IGF—insulin-like growth factor; NGF—nerve growth factor.

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endothelial cells and induces the production of growth factors such as fibroblast growth factors (FGFs) and transforming growth factors (TGF- β), which elicit autocrine functions related to angiogenesis.

The selective entry of circulating leucocytes into different tissue compartments involves the expression of surface structures on endothelial cells. The endothelial cells express intracellular adhesion molecules 1 and 2 (ICAM-1,2) which are members of the immunoglobulin supergene family. They function as ligands for a member of the leucocyte integrin family, LFA-1, present on lymphoid cells, monocytes, and neutrophils. The expression of ICAM-1 (but not ICAM-2) is augmented by exposure of endothelial cells to the inflammatory cytokines IL-1 and TNF α .³ IFN γ also causes a slow increase in ICAM-1 expression. In addition to augmenting ICAM-1 on endothelial cells, IL-1 and TNF α induce de novo expression of other adhesion structures including E-selectin and VCAM-1. The E-selectin molecule is a member of a newly described family containing a lectin domain which determines the molecule's adhesive properties through binding to specific carbohydrate regions. VCAM-1 is the ligand of the very late antigen (VLA-4), an integrin expressed on T lymphocytes.⁴ These molecules, in conjunction with tissue specific endothelial adhesion molecules such as the vascular addressins, will determine the migration of leucocytes at sites of inflammation where IL-1 and TNF α are produced. Induction of E-selectin by IL-1 is short lasting (8–12 hours) compared with VCAM.⁵

At sites of inflammation or during sepsis the release of IL-1 or TNF α induces production of the arachidonate metabolite prostacyclin (PGI₂), and of nitric oxide, both of which are involved in vasodilatation and hypotension.^{6,7} In addition, the antithrom-

botic properties of endothelial cells are profoundly altered by exposure to IL-1 or TNF α which induce tissue type procoagulant activity and suppress the cell surface anticoagulant activity mediated by the thrombomodulin-protein C pathway. IL-1 and TNF α also induce the production of platelet activating factor (PAF) and augment the production of an inhibitor of plasminogen activator, thus inhibiting the dissolution of fibrin polymers.^{8–10}

Endothelial cells are not only influenced by cytokines but also produce several of them, in addition to the growth factors already mentioned. When exposed to inflammatory stimuli—for example, bacterial lipopolysaccharides—endothelial cells produce IL-1 and IL-6 in addition to leucocyte chemotactic and activating cytokines such as IL-8, monocyte chemotactic protein, or monocyte chemotactic and activating factor (MCP/MCAF).¹¹ They can also produce colony stimulating factors (CSFs) such as granulocyte (G) and granulocyte macrophage (GM) CSFs that are involved in cell differentiation. All these cytokines derived from endothelial cells can thus not only regulate the recruitment of leucocytes, their proliferation and differentiation, but also the function of endothelial cells themselves by acting in autocrine circuits.¹² This illustrates the complex bidirectional interactions between cytokines and a given cell type.

Cytokines and mononuclear phagocytes

Macrophage colony stimulating factor (M-CSF) stimulates the production of monocytes and macrophages from bone marrow progenitor cells and acts on mature monocytes and tissue macrophages to stimulate the secretion of plasminogen activator, thromboplastin, and cytokines such as IFN α , TNF α , and G-CSF. It has also been found to induce the tumoricidal, fungicidal, and bactericidal activity of macrophages in culture. M-CSF has been found in numerous cell types such as fibroblasts and endothelial cells. The receptor for M-CSF is the proto-oncogene C-fms which is found on normal macrophages and macrophage related cells.¹³ GM-CSF is also capable of enhancing the clonal development of normal granulocyte macrophage progenitors. It likewise stimulates monocytes and macrophages to release prostaglandin E, plasminogen activator, or cytokines. GM-CSF has a wide distribution in various tissues and is released after stimulation by other cytokines or bacterial products.¹⁴

Mononuclear phagocytes respond chemotactically to a number of cytokines (table 2). Two related families of cytokines, the α and β chemokines, are released by several types of cells after stimulation by lipopolysaccharide, IL-1 or TNF.¹⁵ The β chemokines (for example, MIP-1, MCP-1, RANTES) are chemotactic for monocytes and macrophages while α chemokines (for example, IL-8, neutrophil activating protein (NAP-2)) are chemotactic for neutrophils, lymphocytes, or

Table 2 Some chemotactic factors for neutrophils and other leucocytes

	Cell type		
	Neutrophil	Monocyte/ macrophage	Lymphocyte
Complement peptides			
C5a, C5a des ay	+	+	–
Lipids			
Leukotriene B4	+	+	–
Platelet activating factor	+	+	–
Formyl peptides			
f-Met-Leu-Phe	+	+	–
Cytokines			
GM-CSF	+		
TNF α and TNF β	+	+	–
TGF- β	+		
α chemokines			
IL-8 (NAP-1)	+	–	+
NAP-2	+	–	–
β chemokines			
MCP-1	–	+	–
RANTES	–	+	+
Modified proteins:			
e.g. denatured albumin, haemoglobin, peptide fragments from elastin, collagen	+	+	+

For definitions see footnote to table 1.

both. Other cytokines that attract mononuclear phagocytes are transforming growth factor (TGF)- α , TNF α , and TNF β . Like neutrophils, monocytes and macrophages also show chemotactic responses to microorganisms and factors such as formyl-Met-Leu-Phe, C5a, leukotriene B₄, platelet activating factor (PAF) and denatured proteins.

Macrophages are essential elements of the inflammatory response.¹⁶ They have the capacity to secrete a large number of molecules that regulate the immune response, release components of the complement cascade, procoagulants and anticoagulants, proteases, lipases, DNAses, metalloproteinases, reactive oxygen intermediates (ROI) such as superoxide and hydrogen peroxide, and reactive nitrogen intermediates such as nitric oxide.¹⁷ Uncontrolled secretion of any of these mediators can produce extensive tissue damage. These activities of mononuclear phagocytes are tightly controlled by the balance of the activating and inhibitory signals received. Interferon γ , produced by the T helper cells TH1 lymphocytes, is the principal macrophage activating factor.¹⁸ Primed macrophages are characterised by the increased surface expression of class II major histocompatibility complex (MHC) molecules, LFA-1 molecules, and by an increased capacity to release ROI. In the presence of lipid A, a component of lipopolysaccharide, primed macrophages secrete large amounts of cytolytic proteinases, IL-1, and TNF α . While IFN γ increases IL-1 and TNF production by macrophages, other cytokines such as IL-4 or IL-10 produced by TH2 lymphocytes can profoundly inhibit the production of these inflammatory proteins. IL-4 represses not only production of IL-1¹⁹ but also of the metalloproteinases gelatinase A and B.²⁰ IL-4 also enhances the transcription and release of inhibitors such as IL-1 receptor antagonist.²¹ Signals which suppress activation include TGF- β , PGE₂, or IFN β , depending on the particular form of macrophage activation or type of function examined.

It is clear therefore that cytokines, acting alone or in concert, are capable of exerting a considerable effect on which components of the vast repertoire of macrophage functions are expressed in any given situation.

Table 3 Cytokines secreted by T lymphocytes

	CTL	TH1	TH2
IFN γ	++	++	-
IL-2	+	++	-
TNF β	+	++	-
GM-CSF	++	++	+
TNF α	+	++	+
IL-3	+	++	++
IL-4	-	-	++
IL-5	-	-	++
IL-6	-	-	++
IL-10	?	-	++

CTL—cytotoxic T lymphocytes (CD8); TH—T helper lymphocytes. For other definitions see text.

Cytokines produced by TH1 or TH2 lymphocytes (table 3)

T cells play a central role in controlling immune responses. They recognise antigen(s) complexed with MHC molecules or antigen presenting cells, through their T cell antigen receptors (TCR) which was composed of two different (heterodimer) glycoprotein chains. The intracellular signalling pathway which activates resting T cells is transduced by a group of molecules physically linked to the TCR (the CD3 complex) and other molecules such as CD2, CD4, and CD8. Binding to the TCR and activation of related molecules induces the expression of a number of genes including growth factor receptors (for example, IL-2 receptor).²² Several cytokines are involved in T cell activation and proliferation. These may be divided into cytokines which have a direct mitogenic effect (such as IL-2, IL-4, IL-7), and cytokines which are required to obtain optimal stimulation but do not directly induce proliferation and expansion of T cells (such as IL-1 α/β , IL-6, TNF α , TNF β , and GM-CSF).

Studies of CD4+ T cell clones have resulted, at least in mice, in the differentiation of T helper (TH) cells into two subsets: TH1 lymphocytes produce IFN γ , IL-2, and TNF β ; TH2 lymphocytes secrete IL-4, IL-5, IL-6, and IL-10.²³ Most human T cell clones do not fit into the TH1 and TH2 categories, although representatives of these two subtypes do exist. The molecules inducing the differentiation of TH precursors into TH1 or TH2 cells are not known but may be related to the nature of the antigenic trigger. Tuberculin favours a TH1 type of response while allergens favour a TH2 differentiation. TH1 induces delayed hypersensitivity responses and favours the activation of specific cytotoxic CD8 lymphocytes and natural killer (NK) cells by the production of IL-2 and IFN γ . The IFN γ and TNF β released by TH1 enhance the bactericidal and tumoricidal activity of macrophages. TH2 cells induce a strong differentiation and proliferation of B cells by their production of IL-4, IL-5, and IL-10. IL-4 allows the production of IgE whereas IFNs from TH1 block IgE production.²⁴ IL-2 and IFN γ are also capable of inducing proliferation and differentiation of B cells but favour the production of IgG2a.

TH2 lymphocytes can control TH1 lymphocytes and vice versa. TH2 cells therefore inhibit the production of cytokines by TH1 cells and NK cells²⁵ by releasing IL-4 and IL-10. TH1 lymphocytes, by releasing IFN γ , block the proliferation and differentiation of the basophils and mastocytes or the eosinophils controlled by the TH2 production of IL-3, IL-4, IL-5, and IL-10.²⁶

Cytokines and neutrophils

Because of the short half life of the circulating neutrophils the bone marrow must continually produce cells to maintain a state of equilibrium. The mechanism allowing this rapid and specific increase of mature cells was

understood recently when the cytokines modulating proliferation of the myelopoietic cells were purified. After commitment of progenitors cells into the granulocyte lineage, G-CSF and GM-CSF play a predominant part in the proliferation and differentiation as well as in the activation of the mature cells.²⁷ G-CSF is a 19 kDa polypeptide whose production is not constitutive and requires cell activation. The production of G-CSF by monocytes is activated by endotoxins or by GM-CSF, IL-3, and IL-4; TNF α and IL-1 activate fibroblasts and endothelial cells to release G-CSF, and in vitro mitogens activate T lymphocytes. The number of G-CSF receptors increases during neutrophil maturation. G-CSF alone is a strong stimulus of neutrophil function, particularly increased chemotaxis, production of superoxides, and antibody dependent cell cytotoxicity.

GM-CSF is a glycoprotein composed of 127 amino acids. It is encoded by a gene located on the long arm of chromosome 5 and produced by various cell types in response to numerous stimuli. IL-1 stimulates its production by endothelial cells and the thymic epithelium, while IFN γ enhances its production by monocytes and T lymphocytes.²⁸ The recent finding that it is released (along with IL-3) by mast cells in response to activation of the IgE receptor is of importance since this mechanism may be involved in hypersensitivity reactions.²⁹ GM-CSF promotes the long term survival of haemopoietic progenitors. It is able to sustain the production of mature granulocytes and monocytes. It is noteworthy, however, that G-CSF and M-CSF potentiate the action of GM-CSF on granulopoiesis and monocytopoiesis respectively. An increase of about three fold in the number of circulating leucocytes is noted when GM-CSF is administered for three days at a dose of 8 μ g/kg. This leucocytosis results from an increase in neutrophils, eosinophils, and monocytes, whereas the absolute number of lymphocytes and of their subsets is unaltered. GM-CSF is also a potent activator of mature neutrophils. It therefore activates their cytotoxic activity, their synthesis of PGE₂, their production of superoxides, and the expression of the adhesion glycoproteins located on their cell membrane.^{30 31} GM-CSF has a priming effect and increases the production of platelet activating factor (PAF) following stimulation with FMLP or TNF α , or after phagocytosis.²⁷ This may be an important observation in view of the crucial role of PAF in inflammation.

IL-8 was initially detected as a selective in vitro chemoattractant of neutrophils, but not of monocytes.³² This was soon followed by data showing that IL-8 could also be isolated on the basis of its capacity to cause the release of lysosomal enzymes from neutrophils. IL-8 increases adherence of neutrophils to unstimulated endothelial cells, to fibrinogen, and to matrix proteins; it increases granulocyte cell surface expression of mac-1 (the receptor for the inactivated complement component C₃bi) and of complement receptors; it

increases the production and release of leukotriene B₄ (LTB₄) and 5-hydroxy-eicosatetraenoic acid (5-HETE). The stimulation of superoxide anion and hydrogen peroxide production by neutrophils with recombinant IL-8 remains controversial.¹⁵ IL-8 is the best known member of the α chemokine family. In response to IL-1 or TNF α , IL-8 mRNA is expressed in a number of human cell types including peripheral blood mononuclear cells, dermal fibroblasts, endothelial cells, and keratinocytes.^{33 34} Stimulation of T lymphocytes with lectins also induces IL-8 production. About 20 000 receptors per neutrophil have been detected with a single type of high affinity binding, while T lymphocytes only have about 300 receptors per cell. IL-8 downregulates its own receptor expression. More than 90% of IL-8 receptors are internalised within 10 minutes of IL-8 binding. Following internalisation the IL-8 molecule is slowly degraded by lysosomal enzymes and the digested form is exocytosed from the cell. By liberating the receptors from IL-8, the downregulated IL-8 receptors are quickly re-expressed on the cell membrane. This rapid turnover of IL-8 receptors may be important for the chemotactic responses of neutrophils.³⁵

The other members of the α chemokine family show considerable homology to IL-8. The growth regulated gene product (GRO) or the melanoma growth stimulating activity (MGSA) appear identical. They can both stimulate the growth of fibroblasts and degranulate neutrophils. They compete almost as well as IL-8 for binding to the IL-8 receptor. Platelet factor 4 (PF4), derived from the α granules of platelets, is reported to have a weak chemoattractant activity for neutrophils and monocytes, but a more potent action for fibroblasts. PF4 also promotes the proliferation of fibroblasts, the synthesis of glycosaminoglycan by chondrocytes, and neutrophil degranulation, but not the oxidative burst. Platelet basic protein (PBP) is also derived from the α granules of platelets. After proteolytic removal of the first nine amino acids, PBP becomes the "connective tissue activating protein" (CTAP) reported to stimulate the proliferation of synovial cells and the production of glycosaminoglycan. Loss of four more amino terminal amino acids from CTAP-III yields β thromboglobulin (β TG) which is a potent chemoattractant for fibroblasts with weak effects on neutrophils. Proteolytic removal of another 11 amino acids from β TG yields a somewhat more potent molecule, 70 amino acids in length, known as "neutrophil attractant 2" (NAP-2) which is 100 times less potent in attracting neutrophils than IL-8 or C5a.¹⁵

Cytokines and eosinophils

Eosinophil development is strictly dependent on T cells. It does not take place in experimental animals deprived of T cells, and it is severely reduced in patients with T cell deficiencies such as AIDS. The critical T cell

function is dependent upon the production of the haematopoietic growth factors IL-3, GM-CSF, IL-4, and IL-5. IL-5 induces the proliferation and differentiation of haematopoietic progenitors of eosinophils, and only IL-5 appears to be specific for the eosinophilic lineage. IL-3, GM-CSF, and IL-4 can also favour the proliferation of eosinophils; IL-5, IL-3, and GM-CSF increase the viability of eosinophils; IL-5 and IL-3 enhance the cytotoxic activity of eosinophils against schistosomes; GM-CSF increases eosinophil antibody dependent cytotoxicity as well as their phagocytic capacity³⁶; and IL-4 increases the expression of IgE receptors on eosinophils while decreasing their IgG receptors.

LTB₄ is a powerful chemotactic and chemokinetic substance for eosinophils (table 2). PAF may be an even more powerful eosinophil chemoattractant. IL-5 is also chemotactic for eosinophils³⁷ and IL-5 secretion, by infiltrating mononuclear cells, may explain the simultaneous delayed maximal accumulation of mononuclear cells, and eosinophils 24–48 hours after allergen challenges of the skin.³⁸

Cytokines and mast cells

Mast cells are derived from multipotential stem cells in the bone marrow. IL-3 stimulates the proliferation and differentiation of haematopoietic progenitor cells to generate mast cells, as well as neutrophils and macrophages. Mast cells are distributed in connective tissues, often adjacent to blood vessels and beneath epithelial surfaces. They do not circulate, are long lived, and retain the capacity to proliferate. IL-3, IL-4 and, perhaps, IL-10 produced by the TH2 cells appear to be important for this local proliferation, as animals depleted of T cells or treated with antibodies against IL-3 or IL-4 do not have an increased number of mastocytes following an helminthic infection.³⁹

The release of inflammatory mediators such as histamine or leukotriene C₄ by mastocytes when exposed to known activators such as C_{5a} or antibodies against IgE receptors can be enhanced by IL-3, IL-5, IL-8, or GM-CSF. The latter cytokines could also enhance the release of other secretagogues such as the eosinophil major basic protein, neuropeptides (for example, substance P), and proteins released from neutrophils.³⁹

Mast cell lines produce IL-4 and GM-CSF.⁴⁰ Peritoneal mast cells contain a significant amount of preformed TNF α that can be released after stimulation. After IgE receptor crosslinking proinflammatory cytokines are rapidly produced (IL-1, TNF, IL-8) while other cytokines that can amplify the allergic reaction such as IL-3, IL-4, IL-5, or GM-CSF are released after an interval.⁴⁰ Mastocytes therefore produce chemotactic cytokines and cytokines with biological properties similar to those produced by TH2 lymphocytes.

Cytokines and fibroblasts

Alveolar macrophages are capable, when appropriately activated, of releasing a number of potent fibrogenic growth factors such as platelet derived growth factor (PDGF), insulin like growth factor (IGF), acidic and basic fibroblast growth factors (aFGF, bFGF), and transforming growth factor β (TGF- β).⁴¹

PDGF was initially isolated from *a* granules of platelets but is secreted by several cells including fibroblasts, smooth muscle cells, endothelial cells, and activated macrophages. It is a 30 kDa dimer with two subunits, A and B, linked by a disulphide bond. PDGF attracts mesenchymal cells and stimulates them to enter the growth cycle by entering the G₁ phase of the cell cycle. The PDGF B chain gene is the *c-sis* proto-oncogene. With human fibroblasts which contain three to four times more type B than type A PDGF receptors, PDGF AB and PDGF BB are found to be potent mitogens while PDGF AA induces only a limited mitogenic stimulation.⁴² The half life of PDGF in plasma is very short because of its rapid binding to one or more other proteins: α_2 -macroglobulin has been implicated as the major binding protein for PDGF. Rat alveolar macrophages secrete a protein able to inhibit PDGF by a similar mechanism.⁴³

IGF-1 or somatomedin C was previously called "alveolar macrophage derived growth factor." It acts late in the G₁ phase of the growth cycle to take mesenchymal cells stimulated by PDGF or fibronectin through to the S phase and subsequent proliferation.⁴⁴

Acidic and basic fibroblast growth factors (FGFs) are structurally related peptides characterised by their high affinity for heparin. Both types are mitogenic for mesenchymal cells and are among the most potent known inducers of neovascularisation. They are mitogenic and possibly chemotactic for endothelial cells and can induce synthesis of plasminogen activator. They have been isolated from several tissues including brain and kidney. FGFs have been identified immunohistochemically in peritoneal macrophages.⁴¹

There are several species of TGF- β coded by different genes. Mammals express three types of TGF- β . TGF- β 1 mRNA is predominantly expressed in haematopoietic cells, TGF- β 2 transcripts are found mainly in developing bone, and TGF- β 3 mRNA is synthesised by various cell lines chiefly of mesenchymal origin. TGFs are synthesised as larger secretory precursor polypeptides. The pro-TGF- β dimerises by forming a disulphide bond, then a proteolytic cleavage occurs to give the mature TGF- β dimer.⁴⁵ Activation of the mature form occurs *in vitro* by treatment with extreme pH, heat, proteases such as plasmin or cathepsin D, or glycosidases, or both. The elucidation of the *in vivo* activation process will be important in understanding the target specificities of TGF- β .

The growth regulatory effects of TGF- β are dependent on the cell type and presence of additional growth factors. Thus TGF- β

can inhibit growth of fibroblasts or epithelial cells in soft agar, but can stimulate the growth of fibroblasts in the presence of other growth factors.⁴⁶ TGF- β is a regulator of extracellular matrix (ECM) deposition and attachment of cells to the ECM. The structural proteins that constitute the ECM such as fibronectin, collagen, chondroitin/dermatan sulphate proteoglycans, and glycosaminoglycans are all induced by TGF- β .⁴⁷ It also induces the synthesis and secretion of protease inhibitors such as tissue inhibitor of metalloproteinases and plasminogen activator inhibitor which are involved in the degradation of ECM.^{46,48} Furthermore, TGF- β causes a decrease in the corresponding enzymes involved in the breakdown of the ECM. In vivo studies have shown that TGF- β accelerates wound healing and induces granulation tissue, particularly because of its stimulation of the production of ECM proteins.

IL-1 α and IL-1 β are either not involved in the induction of mesenchymal cell proliferation or act indirectly. Recent studies suggest a mechanism by which IL-1 may induce proliferation by inducing fibroblasts to secrete a PDGF A chain homodimer which stimulates cells to enter the cell growth cycle. Further amplification of the system during longer culture periods may be the result of PDGF upregulation of IL-1 receptors on fibroblasts of IL-6, IL-8, TNF α , MCP-1, and a number of colony stimulating factors.⁴⁹

TNF α has been reported to act as both a growth inducer and inhibitor; at low concentrations it stimulates fibroblast proliferation and at higher concentrations it blocks growth triggered by serum or other cytokines. The role of IL-1 and TNF α in the production of collagen is questionable.⁴⁶ Some reports state that TNF α enhances collagen synthesis while other reports show suppression. Studies have suggested that TNF α is the critical mediator responsible for the pathogenesis of pulmonary fibrosis induced by bleomycin. However, these studies do not prove that TNF α is a direct mediator of fibrosis, inducing fibroblast proliferation or collagen synthesis, but rather that the role of other mediators may be dependent on it. TNF α may also cause diffuse alveolar damage.⁴¹

The release of macrophage derived growth factors is also triggered by other lymphokines derived from CD4+ helper T cells. This can be mimicked in vitro by culturing lung macrophages from control animals with IFN γ . Peritoneal macrophages and blood monocytes can also be stimulated by IL-2 to express genes encoding TGF- β and PDGF B.

Inflammatory cytokines and their inhibitors

IL-1 receptor antagonist (IL-1ra) is a specific inhibitor of IL-1 that binds to the IL-1 receptor types I and II but fails to stimulate target cells.^{50,51} It was originally found in vivo as a 22 kDa IL-1 inhibitor in the urine of febrile patients with monocytic leukaemia. IL-1ra was also discovered in the supernatants of

human monocytes cultured on immune complexes or adherent immunoglobulin (IgG). Alveolar macrophages secrete large amounts of IL-1ra when they adhere on plastic in the presence of lipopolysaccharide or with IL-4.^{21,52} IL-1ra affinity for IL-1 receptors is equal to that of IL-1. The inhibition of IL-1 induced biological responses in T cells, fibroblasts, and chondrocytes in vitro requires 10–100 fold excess amounts of IL-1ra. This is probably because the target cells are exquisitely sensitive to minute amounts of IL-1 as only 2–5 % of IL-1 receptor needs to be occupied to obtain a complete biological response. The studies published so far indicate that IL-1ra has potent in vivo biological effects in several animal models of endotoxin induced lung injury or shock, as well as graft versus host disease.⁵¹

Proteins representing the extracellular domain of the IL-1 receptors have been generated by recombinant DNA technology. Extensive work is in progress to establish whether this material is shed under physiological or pathophysiological conditions, or both. It is found that the recombinant soluble IL-1 receptors have an inhibitory role of various IL-1 actions in vitro. This would add to the list of potentially useful inhibitory proteins.

The two soluble forms of TNF α receptor (55 and 75 kDa) are inhibitory proteins capable of forming stable complexes with TNF α and TNF β , their affinity being higher for TNF α than for TNF β .⁵³ The TNF inhibitory proteins block TNF bioactivities such as collagenase and PGE₂ production by synovial cells, or the neutrophil respiratory burst induced by TNF α .⁵⁴ The proteolytic cleavage of the two TNF α receptor molecules is probably an important mechanism for controlling inflammation. The soluble TNF α inhibitors RI (55 kDa) and RII (75 kDa) are found in small amounts (1–3 ng/ml) in the serum of healthy persons as well as in synovial fluids, or in smaller amounts in bronchoalveolar lavage fluid. The possible imbalance between TNF α and its inhibitors may be important for the clinical prognosis of severe disease. The timing of the release of IL-1 or TNFs and their natural inhibitors may be crucial. The regulation of IL-1 and IL-1ra at the transcription and at the translation level needs to be better understood. Other truncated forms on the extracellular domain of cytokine receptors are potential cytokine inhibitors. Such molecules have been shown for IL-1 (p55), IL-4, IL-7, and IFN α . All receptors are not inhibitors: such is the case for the soluble IL-6 receptor.⁵⁵

Concluding remarks

Cytokines stimulate the release of a large number of factors which may interact in a series of complex networks. Their action is chiefly local involving paracrine (effects on neighbouring cells) and even autocrine (effects on the same cell) functions. Several cytokines appear to have similar actions on either cell maturation, differentiation, or

activation. Some cytokines exert more specific functions: as growth factors such as the various CSFs; in chemotaxis such as the chemokines; and in inflammatory processes such as IL-1 and TNFs. Others including IL-6 or TGF- β are more active in repair processes.^{56,57} However, even molecules which have a clear primary role can exhibit other functional activity. A good example is TGF- β which is mainly involved in repair but has, in addition, both immunosuppressive and pro-inflammatory properties.

The biological effects of cytokines are determined by factors within the local environment that may activate a latent molecule (for example, TGF- β), by the target cell type, by the state of maturation or activation of these target cells which determines the number of receptors, and by the role of other growth factors or antagonists. Several cytokines may have effects which are antagonistic to the effects of other cytokines such as IFN γ and IL-2 from TH1 lymphocytes and IL-4, IL-6 or IL-10 from TH2 cells. The balance of effects of these two groups of cytokines will not only determine the type of immunoglobulin produced by B cells, but will activate or inhibit numerous functions of macrophages, eosinophils, mastocytes, or mesenchymal cells (for example, the release of TNF α by macrophages is enhanced by IFN γ but is inhibited by IL-4 or IL-10). A better understanding of their finely tuned interrelationships may help in the control and prevention of a persistent inflammatory response. There also exist several specific inhibitors of cytokines with anti-inflammatory properties such as the IL-1 receptor antagonist which can compete with IL-1 at the receptor level, or soluble receptors of cytokines such as the TNF receptors; the mechanisms of their cleavage, which allows the binding and the inhibition of their corresponding cytokines remain poorly understood. These inhibitors may play a crucial role to limit the extent of inflammation.

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