# THE ROLE OF CYTOKINES IN ASTHMA AND RELATED OCCUPATIONAL LUNG DISEASES: AN OVERVIEW

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## Abstract

Cytokines are potent regulatory molecules involved in cell to cell communication. They have a clear role in the development of inflammation that is seen in both asthma and occupational lung diseases. The eosinophilia that is characteristic in the airways in asthma, is predominantly regulated by the cytokine, interleukin 5. It is thought to be such an important mediator that attempts have been made to develop drugs to alter it's function. In many of the occupational lung diseases however, the major inflammatory cell is the neutrophil. Here, it is the cytokine, interleukin 8 that is believed to influence neutrophil accumulation.

## 1. General Introduction

Cytokines are small, non antigen specific polypeptide or glycoprotein intercellular regulatory molecules. They can be regarded as a mechanism for cell to cell communication and encompass those families of regulators known as growth factors, colony stimulating factors, interleukins, lymphokines, monokines and interferons.

Cytokines are very stable structures ranging in size from a molecular weight of 6000 to 60,000. They contain protective carbohydrate and intra-molecular disulphide bonds which appear to increase solubility, stability and protease resistance (Nicola, 1994).

### 1.1 Actions of cytokines

Cytokines, under the correct stimulation, can be produced from any cell. However, they are usually released from several nucleated cells, such as B and T-lymphocytes, monocytes and epithelial cells. Their regulatory actions extend to both self and other cells mediating a host of immunological and non-immunological responses. Cytokines effect growth, proliferation and functions of all haemopoetic cells (T and B lymphocytes, monocytes, neutrophils and eosinophils) and some have stimulatory properties on non-haemopoetic cells (endothelial cells, epithelial cells, mast cells, hepatocytes, megakarocytes, oligodendrocytes and connective tissue cells).

Various stimuli, e.g. hormones, endotoxins and other endogenous substances, can activate release of cytokines. Once released, cytokines usually act in either an autocrine or paracrine fashion. However, there are exceptions, transforming growth factor and macrophage-colony stimulating factor can occasionally act in an endocrine manner to produce systemic effects.

Cytokines are potent molecules, with activity at concentrations as low as $10^{-10}$ to $10^{-13}$ mol/l (Oppenheim et al, 1993). They mediate their effects by binding with high affinity to specific receptors (Ka = $10^{-9}$ to $10^{-12}$ M). Often occupancy of less than 10% of the receptors on a cell surface can cause activation of the cell (Oppenheim et al 1993). Radio-labelling shows that cytokine receptors on a cell can be absent or only present in low numbers. However, these receptors are induced by cytokine stimulation, consequently increasing in density during cell activation.

### 1.2 Diversity of cytokines.

Cytokines have a widely diverse nature. A particular cytokine can be produced by a whole variety of different cell types, for example, interleukin 8 (IL-8) is secreted by monocytes, endothelial cells, epithelial cells, fibroblasts and chrondocytes (Male 1993). In addition to this, a single cytokine may have the ability to produce a range of effects on different cell types. This is represented by interleukin 4 (IL-4) which can cause proliferation of T-cells, stimulate mast cell growth, aid synthesis of IgE and IgG and inhibit the synthesis of IgM, IgG3, IgG2a and IgG2b by B-lymphocytes (Male 1993).

Several reasons have been postulated for this characteristic of cytokines. It is possible that the same cytokine receptor uses different intracellular signalling pathways in different cells. Alternatively, the cytokine binding part of the receptor may associate with different co-molecules on the surface leading to different signalling pathways (Nicola et al 1991). However, if these differing actions are possible then local action of a cytokine is preferable. Mechanisms are present to achieve this: cytokine producing cells are often physically located adjacent to responder cells; only small quantities of cytokine are secreted; and responder cells have the ability to destroy cytokines to prevent further action.

Also, it has to be taken into consideration that a cell is unlikely to be exposed only to a single cytokine at any one time, and very few biological responses are mediated by a single cytokine. Therefore, the possibility for synergism or antagonism of cytokine effects exists. Lee et al (1984) showed that interferon gamma (IFNγ) potentiated the cytotoxicity of tumour necrosis factor (TNF) to tumour cells, indicating a synergistic action. IFN also opposes IL-4 mediated synthesis of immunoglobulin subclasses in B-cells, so it can act as an antagonist (Snapper et al. 1988).

Cytokines also have the capacity to modulate the expression of receptors for other cytokines, e.g. TNF can stimulate T cells to up-regulate receptors for interleukin 2 (IL-2) and

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IFNγ (Kaye et al. 1984). Down regulation is less well documented although, Holtman et al (1987) showed interleukin 1 (IL-1) mediated decrease in TNF receptors.

## 2. Cytokines and Inflammation

### 2.1 The inflammatory response

A number of respiratory diseases such as asthma and some occupational disorders are characterised by chronic inflammation. The inflammatory response is identified by changes in the microcirculation. There is an increase in blood flow and an increase in permeability of the microvasacular endothelium to inflammatory cells and plasma proteins. This enables them to leak out into the extravasacular compartment establishing an inflammatory exudate. Accompanying this is migration, margination and firm adhesion of leukocytes (including neutrophils, monocytes and eosinophils) to the vascular endothelium, resulting in accumulation in the extravascular tissues.

It is known that cytokines can be produced in response to a stimuli known to cause inflammation, e.g. exogenous bacteria, endotoxin, fungi and immune complexes. This was demonstrated by the release of IL-1 and TNF from monocytes and tissue macrophages after stimulation with an inflammatory agent. Tracey et al (1986) found that administration of recombinant human TNF induced a state of fatal shock with diffuse pulmonary inflammation in rats. Stephens et al (1988), with the same agent, caused an increase in vascular leakage, capillary congestion and neutrophil accumulation in the lungs of guinea pigs. If there is neutrophil accumulation then TNF can also augment the phagocytic and cytotoxic activities of neutrophils (Shalaby et al., 1985) and IL-1 and TNF can stimulate endothelial cells to promote neutrophil adherence (Pohlman et al., 1986).

### 2.2 Mediators of inflammation and their regulation

Cytokines also regulate the mediators of inflammation-histamine and arachidonic acid derivatives. Presently there is little information on the role of cytokines with regards to other inflammatory mediators such as kinins and complement. Histamine is synthesised and stored in mast cell and basophil cytoplasmic granules. It causes vasodilation, enhanced vascular permeability, smooth muscle contraction and mucus production. Haak-Frendacho et al (1988) showed that granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin 3 (IL-3) cause release of histamine from basophils. IL-1 can also promote release of histamine from basophils and mast cells (Hazuda et al 1990).

A vast number of pro-inflammatory molecules are derived from arachidonic acid (figure 1). The cyclo-oxygenase pathway produces prostaglandins, thromboxanes, platelet activating factor and prostacyclin. Prostaglandins E2 and prostacyclin are vasodilators and bronchodilators, with prostacyclin also inhibiting platelet aggregation. Prostaglandin D2, F2 and thromboxane A2 are vasoconstrictors and bronchoconstrictors.

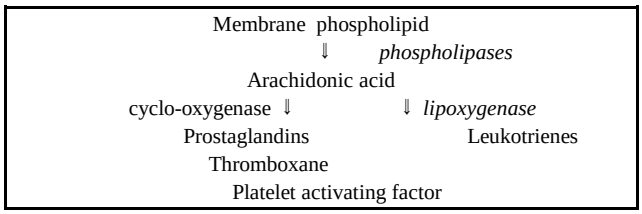


Figure 1: The arachidonic acid pathway

Thromboxane $A_2$ can also cause aggregation of neutrophils and platelets. Platelet activating factor causes bronchoconstriction, increased vascular permeability and is chemotactic for inflammatory leukocytes.

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The lipoxygenase pathway produces leukotrienes, formed by the action of a calcium dependent 5-lipoxygenase enzyme on arachidonic acid. Leukotriene $LTB_4$ acts as a chemotactic factor for leukocytes (neutrophils, eosinophils and monocytes) and promotes their aggregation, adhesion and degranulation. $LTC_4$, $LTD_4$ and $LTE_4$ are leukotrienes which increase vascular permeability, mucus secretion and airway contraction (Henderson et al, 1987).

The cytokines IL-1 and TNF demonstrate enhancement of arachidonic acid metabolism through activation of phospholipase $A_2$, thereby increasing the number of inflammatory mediators (Chang et al 1986; Clark et al 1988). Dinarello et al (1993) showed that IL-1 also stimulates the synthesis of PGE2 in monocytes, TNF is believed to act synergistically in this process (Elias et al 1987). IL-1 stimulates production of PGF2 and PGI2 (Albrighton et al 1985). A positive feedback mechanism appears to occur with $LTD_4$ and $B_4$ augmenting IL-1 production by monocytes. (Rola-Pleszczynski et al 1985).

### 2.3 Leukocyte regulation

Inflammation is, as already mentioned, associated with an influx of various leukocytes. Cytokines are involved in the control of leukocyte infiltration itself. Interleukin 5 (IL-5) is a chemotactic factor for eosinophils (Yamaguchi et al 1988), and TNF has been found to be a chemotactic factor for monocytes and polymorphonuclear leukocytes. (Minget et al 1987).

In the event of antigen recognition, leukocytes marginate and adhere to the endothelium. The rapid transition from non-adherent to adherent states is dependent upon expression of cell surface markers and adhesive receptors. IL-1, IL-4, TNF and IFN have the ability to promote induction and upregulation of adhesion molecules on leukocyte surfaces and the endothelium.

Mulligan et al (1995) demonstrated that TNF and IL-1 from alveolar macrophages caused upregulation of ICAM-1 (an adhesion molecule of the immunoglobulin supergene receptor family) and ELAM (a selectin adhesion molecule)

in the lungs of rats. TNF has also been shown to upregulate another adhesion molecule from the immunoglobulin supergene receptor family - VCAM-1 (Kips 1993; Wellicome et al 1990). IFN enhances ICAM-1 expression (Dustin et al 1988) and IL-4 increases VCAM-1 expression on vascular endothelium (Thornhill et al 1990). MAC-1 (an integrin adhesion molecule) on eosinophils was upregulated by IL-3, IL-5 and GM-CSF (Walsh et al 1990) and on neutrophils by IL-8 (Diamond et al 1990).

## 3. Cytokines in Asthma

### 3.1 Eosinophilia

Studies indicate that airway inflammation is present in patients with the mildest to most severe forms of asthma (O'Byrne et al 1991). Continuous inflammatory activity can be detected even in clinically stable asthmatic patients. Airway eosinophilia is a well established inflammatory component in asthma and it is suggested that it plays a major role in mediating the extensive epithelial damage associated with this disease.

Like all leukocytes, eosinophils develop from the bone marrow and migrate to tissues where they become activated (Devos et al 1995). Eosinophils are believed to have a protective function in the body due to a phagocytic mechanism and through release of cytotoxic proteins from their granules. These cytotoxic proteins could possibly be the underlying mechanism of eosinophil mediated damage to the epithelium in asthma.

There are 4 principal proteins of this group - major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP) and eosinophil derived neurotoxin (EDN). MBP, EPO and ECP all have the potential to cause epithelial damage. MBP is involved with bronchial hyperreativity and bronchospasm, and MBP and EPO can activate mast cells, causing release of histamine and bronchospasm.

It appears that the relative content of cytotoxic proteins within eosinophils varies depending on the source. Normal eosinophils tend to contain 10µg of these proteins/$10^6$ eosinophils (Venge et al 1991). However, there is evidence to prove the existence of hypodense eosinophils. These eosinophils appear vacuolated and contain smaller sized granules compared to normal density eosinophils (Moqbel, 1994). Hypodense eosinophils show increased cytotoxic properties (Sarmiento et al, 1995), greater oxygen consumption and are more reactive to a variety of stimuli (Sanjar et al, 1990). Of major importance is that asthmatics have a higher proportion of hypodense eosinophils in their circulation.

The cytokines IL-3, GM-CSF and IL-5 are involved in the regulation of eosinophils. IL-5, due to its important characteristics, will be discussed as a separate entity later. IL-3 and GM-CSF promote early eosinophil growth and differentiation from precursor cells in the bone marrow (Yamaguchi et al 1993). *In vitro* they prolong eosinophil survival and facilitate the conversion of normodense eosinophils to the activated hypodense phenotype. (Sarimento et al, 1995). Owen et al (1987) showed that eosinophils cultured with GM-CSF showed a dose dependent enhanced survival, with a 50% maximal viability enhancement at a concentration of approximately 3ρM GM-CSF (Owen et al, 1987). For IL-3 there was also a dose dependent enhanced survival; however, the 50% maximal response was at a lower concentration of 0.1ρM IL-3 (Rothenberg et al, 1988).

IL-3 and GM-CSF also potentiate the eosinophil promoting effects of the cell derived chemotactic factor, platelet activating factor (PAF). Sanjar et al (1990) showed a dose dependent increase in eosinophils in bronchoalveolar lavage (BAL) when guinea pigs were exposed to aerosols of PAF.

### 3.2 The role of interleukin 5

Interleukin 5 is believed to be a prominent cytokine involved in asthma. It is a prime mediator of eosinophilia and induces eosinophil differentiation and proliferation from bone marrow (Clutterbuck et al, 1988; Coffman et al, 1989). Eosinophils appear to migrate into tissues where IL-5 is expressed (Sanderson 1992) and IL-5 shows chemotactic activity towards eosinophils (Yamaguchi et al, 1988). Nevertheless, this activity is relatively weak and the relevance of this characteristic *in vivo* is unclear. However, IL-5 has been shown to prime eosinophils for a more efficient chemotactic response to other chemotactic mediators, such as PAF, $LTB_4$ and IL-8. Also, IL-5 can upregulate adhesion molecule integrin CD11b on human eosinophils with an accompanying increase in adhesion to endothelial cells (Walsh et al., 1990). These phenomena provide possible mechanisms for the eosinophil localisation seen. The actions of IL-5, unlike IL-3 and GM-CSF, are relatively selective for eosinophils and this suggests this cytokine is a critical mediator in the asthma process.

IL-5, like GM-CSF and IL-3, enhances the viability of eosinophils (Rothenberg et al, 1989; Yamaguchi et al, 1988) and mediates conversion of normodense to hypodense eosinophils (Rothenberg et al, 1989). Yamaguchi et al (1988) showed that the enhanced viability with IL-5 was superior to that seen with IL-3 and GM-CSF, with studies indicating that few viable cells were left after 8 days on incubation with GM-CSF however, with IL-5 the viability was extended to over 10 days (Yamaguchi et al, 1988).

### 3.3 Sources of interleukin 5

IL-5 is produced by several different cell types. $CD4^+$ T-helper Lymphocytes ($CD4^+$T cells) have the capacity to elaborate a wide variety of cytokines, including IL-5 (Corrigan et al, 1992). Jeffery et al (1989) showed that the number of activated Tcells correlated with the degree of peripheral blood eosinophilia. There are two types of T

cells - Th1 and Th2. Th1 cells secrete the cytokines IL-2, IFN , and TNF and are the principal effector of phagocyte mediated host defence. Th2 cells secrete IL-4 which stimulates IgE and IgG1 antibody production, IL-5, IL-10 and IL-13. The Th2 cell is mainly responsible for phagocytic independent host defence. Cytokines such as IL-3, GM-CSF are secreted by both cell types. T cells obtained from BAL of subjects with mild atopic asthma show increased RNA expression for IL-5, suggesting a predominance of $CD4^+$ Th2 cells. $CD4^+$ T cells are thought to be a prime source of IL-5 in asthma.

$CD8^+$ T-lymphocytes / Cytotoxic T cells ($CD8^+$ T cells) mediate lysis of virus infected cells and help control viral replication by producing IFN (Fong et al,1990). However, Coyle et al (1995) demonstrated that $CD8^+$ T cells in mice could be stimulated by IL-4 and viral peptide to switch their cytokine producing profiles from $IFN_\gamma$ to IL-5 production, therefore causing eosinophilia. This is a possible explanation of predisposition and exacerbations of asthma with viral infection as the asthmatic lung contains IL-4.

Another source of IL-5 is mast cells. IgE dependent activation of the mast cell caused expression of IL-5 mRNA which was evident by 2hrs and persisted for up to 48-72 hours (Okayama et al, 1995).

Finally, not only do eosinophils respond to IL-5 they also produce and secrete it, thereby acting in an autocrine fashion producing a classic 'vicious circle'. Briode et al (1992) investigated the BAL of asthmatics after allergen challenge and confirmed that eosinophils did indeed express mRNA for IL-5.

**3.4 Regulation of IL-5 production**

IL-4 and IL-2 have been found to enhance the production and secretion of IL-5 from $CD4^+$ T lymphocytes. Swain et al (1990) demonstrated that exposure of naive $CD4^+$ T cells to IL-4 caused precursors to develop predominantly into a population of the Th2 subtype, therefore enhancing secretion of IL-4 and IL-5. Cells developed in cultures in the absence of IL-4 did not acquire the ability to secrete IL-5 and IL-4. It has been suggested that atopic patients may have an increased Th2 cell population due to priming by IL-4 during foetal development however, the mechanisms remain unclear (Romagnani, 1996). IL-2, on the other hand, enhances the production of both Th1 and Th2 cells, thereby increasing the secretion of IL-5 but non-selectively.

Interleukin 12 (IL-12) inhibits production of IL-5 by $CD4^+$ cells. Funkelman et al (1994) showed that administration of IL-12 during the initiation of an immune response led to a change in T cell profile from Th2 to Th1, reducing the secretion of IL-5.

**3.5 Structure of IL-5 and its receptor**

Interleukin 5 is a glycoprotein which is highly homologous between species, the human mature protein consisting of 115 amino acid residues (Milburn et al, 1993). Structures of IL-5 derived from X-ray crystallographic and NMR studies bear a certain homology to those of other cytokines, particularly IL-4 and GM-CSF, which consists of a bundle of four α helices with two over-connecting loops.

Naturally, IL-5 exists as a disulphide linked homodimer (McKenzie et al, 1990) forming an elongated ellipsiodal disc. Dimerisation is through two disulphide bridges connecting Cys 44 of one monomer to Cys 86 of the other. Also, an antiparallel β sheet is formed between residues 32 to 35 on one monomer and 89 to 92 on the other monomer. Investigations based on reduction and alkylation of IL-5 have shown that the monomer has no biological or inhibitory activity and that dimerisation is essential for biological activity (Tsuruoka et al, 1990).

There is a specific IL-5 receptor on eosinophils (Pazdrak et al; Denburg et al, 1991). It belongs to the type 1 cytokine receptor family , which also includes receptors for the cytokines GM-CSF, IL-2 and interleukin 7 (IL-7). It consists of two distinct transmembrane poplypeptide subunits - an α chain (60kDa) and a β chain (130kDa). The β subunit is also common to the GM-CSF and IL-3 receptors, which help explain the similar biological functions of these cytokines.

The α chain is a low affinity IL-5 binding site, with a Kd of $10^{-9}$ M. Association of the α chain with the β chain gives a higher affinity, Kd = $5x10^{-11}$ M. However, the β subuint dose not bind IL-5 in the absence of the α subunit. Studies with eosinophils suggest high affinity binding, indicating that the combined α and β subunits are present (Tavernier et al, 1991).

Differences have been highlighted between hypodense and normodense eosinophils in respect of binding characteristics of IL-5. IL-5 binds to hypodense eosinophils with a five-fold higher Ka than to normodense eosinophils: hypodense Ka = $1.93x10^{-9}$ M; normodense Ka = $0.39\ x10^{-9}$ M. However, there appears to be no significant difference in the number of IL-5 receptors per cell between the two phenotypes. Chichara et al (1993) also showed that the binding capacity for hypodense eosinophils was more prolonged compared to that for normodense eosinophils. This evidence further highlights that IL-5 is an important mediator of eosinophilia in asthma, due to the prominence of hypodense eosinophils in the asthmatic lung.

It has been found experimentally that binding of a cytokine to its α subunit leads to a tight association with the β subunit to transduce signals. The association between the α and β subunits seems to be the most crucial step for signal transduction, as neither the α nor β subunit alone can mediate a signal by IL-5 (Miyajima et al, 1993).

It has been long established that kinases play a crucial role in intracellular signal transduction and many require

tyrosine phosphorylation for activation. Pazdrak et al (1995) demonstrated that IL-5 induces tyrosine phosphorylation and activation of a number of protein kinases in eosinophils. Neither the α or β subunit of the IL-5 receptor has an intrinsic kinase activity. Therefore, there must be additional proteins that mediate a signal from the high affinity binding proteins to the signalling proteins (Sakamaki et al, 1992). A pathway for activation of eosinophils by IL-5 has been proposed by Pazdrak et al (1995). It involves multiple kinases and is known as the "Lyn-Ras-Raf-Mek-Map kinase pathway".

**3.6 Therapeutic strategies in asthma involving IL-5**

As IL-5 appears to be such a potent mediator of eosinophilia in asthma, anti-IL-5 drugs are a logical approach to therapy. There are 3 main stages at which IL-5 action could be blocked : IL-5 production and secretion, IL-5 receptor binding and signal transduction, and IL-5 longevity.

The major cell type producing and secreting IL-5 is the $CD4^+$ T cell. It would be beneficial to inhibit production of the cytokine from these cells; this occurs naturally *in vivo*, where IL-12 causes a switch from Th2 to Th1 cells, thus reducing IL-5 production. Would it be possible to administer IL-12 as an anti-IL-5 drug? Administering cytokines is practical, as in fact, recombinant IL-2 is a recognised drug. It is known as Aldesleukin and is given intravenously for the treatment of metastatic renal cell carcinoma (BMA and RPSGB, 1996). IFNγ is also used, as an adjunct to antibiotics in patients with granulamatous disease (BMA and RPSGB, 1996). However, more research is needed before this avenue of therapy could be employed for asthma.

Receptor binding and signal transduction are major targets for many drugs. Theoretically, it is possible to inhibit the expression of the membrane bound IL-5 receptor α-subunit, or inhibit the interaction between IL-5 and the receptors α and β subunits. More realistic would be development of IL-5 receptor antagonists.

The IL-5 receptor has been investigated and a growing amount of knowledge of its structure and some of the important residues involved in binding have been gained. Isothiozolone can act as a non-competitive IL-5 receptor antagonist by covalently reacting with the sulfhydryl group of a cysteine residue of the IL-5 receptor α chain (Devos et al 1995). It is a specific antagonism as IL-4 and GM-CSF are not affected. However, it is very toxic and alternative analogues of the compound are being investigated.

The use of mutant IL-5 forms has also been tried. Kitamura et al (1989) produced a mutation of the Glu-13 residue of the IL-5 molecule. Their E13Q mutein was found to bind to the IL-5 receptor subunit with equal affinity, but had a much reduced efficacy compared to normal IL-5.

A third compound that is under investigation is the soluble form of the IL-5 α chain receptor (IL-5Rα) itself. Kikuchi et al (1994) isolated the cDNA encoding the soluble form of the IL-5Rα and consequently generated this SIL-5Rα form. SIL-5Rα competitively inhibited the binding of mouse IL-5 to the receptor. However, the affinity of mouse SIL-5Rα was more than ten times lower than the affinity of the mouse IL-5 receptor complexes. In the human, the affinity was only 2 to 3-fold lower. Thus it is acting as a more effective antagonist (Devos et al 1995). The antagonism arises due to the binding of SIL-5Rα to IL-5 which then finds itself unable to bind to the IL-5α chain of the receptor. This is possibly due to steric hindrance or a conformational change of IL-5.

Finally, an approach to reduce the longevity of IL-5 has been examined. Monoclonal antibodies have been raised against IL-5. Chand et al (1992) investigated the effect of administration of murine anti-IL-5 monoclonal antibody on aeroallergen induced allergic late phase bronchial eosinophilia in guinea pigs. It inhibited eosinophil infiltration quite remarkably. However, on subsequent dosing in humans, the body recognised the murine monoclonal anti-bodies as foreign and destroyed them. To prevent this destructive immune response, it was necessary to humanise the murine antibodies. Use of humanised monoclonal antibodies in reducing inflammation has been demonstrated by Isaacs et al (1992), however, in rheumatoid arthritis rather than asthma. Further investigations are still necessary in the asthma field.

## 4.0 Occupational Lung Diseases

Occupational lung diseases encompasses a broad spectrum of disorders from pneumoconiosis due to inert materials to byssinosis due to cotton dust. The role of cytokines in these disease states is likely to be complicated and is still under investigation.

The majority of the disorders share a common characteristic of airways inflammation. For example, the organic dusts, such as cotton and grain dust, all cause an inflammatory response in the lung. When organic dusts are inhaled macrophages are recruited into the lungs. If the macrophages are unable to digest the particles then assistance is necessary and there is release of chemotactic factors, such as IL-1 and TNF, which recruit inflammatory cells. The majority of these recruited cells are neutrophils. Cotton and grain dust induce bronchitis with accompanying neutrophil accumulation (Robinson 1994). This action is not solely specific for organic dusts and can be induced by a variety of agents including metal fumes (Robinson 1994) and inorganic dusts such as asbestos and silicone.

Cotton dust contains many components that could be involved in the inflammatory response seen on its inhalation. Several studies have shown that inhalation of tannin (a water soluble component in leaves and stems of

woody plants) results in influx of neutrophils into the airways (Rohrbach 1994). This invasion of neutrophils can also be seen with inhalation of endotoxin (Rylander 1994).

**4.1 Neutrophilia**

Neutrophils are phagocytic cells that engulf and destroy unwanted materials. They are the first leukocytes that emerge in an inflammatory response due to their highly mobile nature. The neutrophilia observed in inflammation is achieved by two mechanisms - transfer of preformed neutrophils from bone marrow stores to the blood and increased synthesis of neutrophils in the bone marrow.

Neutrophils possess several systems to accomplish the destruction of unwanted material. They undergo a 'respiratory burst' where there is a marked increase in oxygen consumption and the generation of toxic oxygen products (Rang HP et al, 1993). Neutrophils also contain granules that store poteolytic enzymes. These enzymes have several functions including the breakdown of micro-organisms and the cleavage of complement components which can activate the kinin cascade furthering inflammation.

As with eosinophilia, cytokines have a clear role in the control of neutrophil accumulation.

**4.2 The role of interleukin 8**

Interleukin 8 was previously known as monocyte-derived neutrophil chemotactic factor or neutrophil activating peptide-1 due to its actions and it is one of the most potent chemoattractants for neutrophils (Borish et al., 1996). It is believed to be responsible for 40-70% of the neutrophil chemotactic activity produced by bronchial epithelial cells, fibroblasts and pulmonary epithelial cells (Standiford et al., 1993).

Intradermal injections of IL-8 in animals and man produced accumulation of massive numbers of neutrophils (Baggiolini et al., 1995). When nanomolar concentrations of IL-8 were injected into rabbits neutrophilic influx was seen within 30 minutes (Standiford et al., 1993).

As well as being a chemoattractant for neutrophils, IL-8 has several other pro-inflammatory properties. It causes release of neutrophil granule contents, including myeloperoxidase, elastase and β glucurinidase. The presence of IL-8 stimulates expression of cell adhesion molecules and enhances adhesion of neutrophils to endothelial cells and the extracellular matrix through the integrin CD11b/CD18 (Borish et al., 1996; Nicola, 1994). IL-8 is also involved in the formation of bioactive lipids and the respiratory burst in neutrophils (Baggiolini et al., 1995).

**4.3 Sources and regulation of IL-8**

IL-8 was originally isolated from cultures of human blood monocytes. However, it is now apparent that IL-8 can be synthesised and secreted from a variety of immune and non-immune cells including, tissue macrophages, T lymphocytes, neutrophils, human endothelial cells, epithelial cells and smooth muscle cells.

It is believed that IL-8 production in response to an inflammatory stimulus is dependent on various cytokines interactions with alveolar macrophages (Standiford et al., 1993). The macrophages release TNF-α and IL-1 which induce the expression of IL-8 from surrounding cells and thus causes neutrophil accumulation. IL-1 and TNF can induce the expression of IL-8 in virtually all types of cell (Baggiolini et al., 1995). IL-8 synthesis is also induced by lipopolysaccharide and viruses.

**4.4 The structure of IL-8 and its receptor.**

Interleukin 8 belongs to the α chemokine family, which is a group of structurally and functionally related proteins (Borish et al., 1996). There are at present at least 14 distinct members with between 20% and 50% homology in their amino acid sequence (Borish et al., 1996). Chemokines are small peptides (8 to 10 kDa) which are subdivided into two families. This characterisation is based on the positioning of 4 conserved cysteine residues present in the peptide. The C-X-C subfamily, of which IL-8 is a member, has the cysteines separated by a variable amino acid and their primary target is neutrophils. The cysteines in the C-C subfamily are directly attached and their target cells are monocytes and T cells (Borish et al., 1996).

The tertiary structure of IL-8 consists of a double loop configuration with disulphide bonds between the first and third cysteine and the second and fourth (Standiford et al., 1993). These disulphide bonds are necessary for biological activity of the molecule (Baggiolini et al., 1995). Nuclear magnetic resonance spectroscopy shows IL-8 exists as a dimer with the core of the molecule containing 3 antiparallel β strands (Baggiolini et al., 1995). However, unlike IL-5, experiments have shown that the monomeric analogue of IL-8 is the biologically active form (Baggiolini et al., 1995).

The human precursor form of IL-8 contains 99 amino acids. A signal sequence is cleaved on activation to generate several non-glycosylated truncated forms. $IL\text{-}8_{77}$ contains 77 amino acids and is secreted from endothelial cells fibroblasts and epithelial cells (Standiford et al., 1993). $IL\text{-}8_{72}$ is released from mononuclear monocytes and contains 72 amino acids. (Standiford et al, 1993).

Binding studies of human neutrophils have identified selective IL-8 receptors. They are coupled to GTP binding proteins and have Kd = 0.18±0.07nM (Baggiolini et al., 1995). There is rapid internalisation and proteolytic degradation of the ligand when IL-8 binds to its receptor. This is followed by re-expression of the IL-8 receptor on the plasma membrane within 10 minutes (Standiford et al., 1993).

Further studies indicated that IL-8 can be displaced from its receptor by other C-X-C chemokines and it was believed that two types of receptor for IL-8 existed on neutrophils (Baggiolini et al., 1995). This was confirmed by cloning of two cDNAs encoding seven transdomain receptors. One of the receptors has high affinity binding for IL-8 and is known as IL-8R1 or IL-8RA. The other receptor, IL-8R2 or IL-8 RB, has low affinity for IL-8 but a higher affinity for some of the other C-X-C chemokines (NAP-2 and GROα (Baggiolini et al., 1995).

Clark-Lewis et al (1991) discovered that the Glu-Leu -Arg amino terminal sequence was essential for C-X-C chemokine activity. Receptor binding and neutrophil activation was abolished on deletion of this motif. Antagonists to IL-8 have been synthesised by replacing or omitting this sequence, the most successful being R-IL-8 and AAR-IL-8 (Baggiolini et al., 1995).

In conclusion it is clear that cytokines have a major role in the pathology of certain airway conditions. Although, in recent years, the functions of many cytokines have been elucidated, further investigation is necessary before the precise role of cytokines in respiratory diseases is fully understood.

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