Pulmonary immune cells in health and disease: the eosinophil leucocyte (Part I)

C. Kroegel*, J-C. Virchow Jr*, W. Luttmann*, C. Walker* J.A. Warner**

ABSTRACT: Increasing evidence has accumulated to suggest that eosinophils play a key role in the pathogenesis of asthma and other pulmonary diseases by damaging infiltrated bronchial tissue and lung parenchyma.

The first part of this review on eosinophils describes the cellular characteristics and properties of the cell, which help in understanding its role in disease. The article focuses on origin, maturation and differentiation of the eosinophil, its morphological and phenotypical properties, as well as its preformed and newly generated mediators of inflammation. The cause and putative significance of eosinophil heterogeneity in respect to function and density will also be discussed. In addition, the naturally occurring mediators through which eosinophils are activated and communicate with other inflammatory cells are outlined.

The first part closes with new aspects of eosinophil recruitment from the circulation into perivascular tissue, including nonselective and putative selective adhesion mechanisms and chemotaxis.

Over the past 10 yrs, our understanding of the cellular biology, function and pathophysiological role of the eosinophil leucocyte in disease has undergone profound changes. From being widely considered as a modulator of inflammation, the eosinophil is now being regarded as an effector cell in inflammation, which exerts a range of cytotoxic effects on various cells and tissues. It is not only associated with host defence mechanisms in parasitic infestation but is also implicated in the pathogenesis of allergic, immunological and malignant disorders, as well as diseases of unknown origin.

While much still remains to be elucidated about the mechanism of eosinophil-associated inflammation, research in recent years has led to a much better understanding of the possible mechanisms underlying eosinophil activation, recruitment, and the potential role of mediators, such as platelet-activating factor (PAF) and the pluripotent actions of cytokines on this cell. The purpose of this article is to review current knowledge of the biology and immunology of eosinophils with respect to their pathophysiological role in pulmonary disease.

Origin and differentiation

Eosinophils originate from bone marrow precursor cells and differentiate under the control of a number of growth or colony-stimulating factors derived from T-lymphocytes and mesenchymal cells. Three cytokines, granulocyte/macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3) and interleukin-5 (IL-5) are currently recognized in promoting eosinophilopoiesis [1]. Following a differentiation and maturation period of approximately 5 days, the mature eosinophil leaves the marrow and circulates in the peripheral blood with an estimated half-life of 13–18 h (mean blood transient time 25 h) before migrating into the tissue. Eosinophils are distributed in various organs but prefer tissues that interface with an external environment, such as the gastrointestinal, lower genitourinary and respiratory tracts [2]. Although there is a paucity of data on the longevity of the cells, eosinophils, sequestered in tissues, are likely to persist for at least 6 days. However, the life-span of tissue eosinophils may be extended by the effect of systemically and locally released cytokines, such as IL-5 and GM-CSF [3, 4].

Morphology

The most distinguishing morphological features of the eosinophil are a bilobed nucleus and large eosinophilic granules, which stain yellow–pink with eosin and other acid aniline dyes [5]. These elliptical secondary or specific granules possess an electron-dense crystallloid core, that is embedded in a less electron-dense matrix (fig. 1). They appear after the myelocyte stage and may develop from large spherical primary lysosomal granules [6]. Some of these primary granules are thought to develop into
smaller homogeneous dense cytoplasmic bodies containing lysophosphatase [7, 8], which crystallize in vivo and in vitro to form the distinctive bipyramidal Charcot-Leyden crystals [9, 10]. A third type of granule, the so-called small granules, are less conspicuous. They form during the metamyelocyte stage, and increase progressively in number with cellular maturation to become small and less electron-dense homogeneous cytoplasmic structures [11]. All three granules are present in the mature eosinophil [7, 8] (fig. 2), and all contain different constituents (table 1).

Eosinophils also show smooth, elongated, rounded, C-shaped or circular-shaped membrane-bound cytoplasmic structures, that are generally empty but are thought to play a role in the extrusion processes of the cell. They were previously referred to as microgranules [12], but are best identified as vesiculotubular structures [13, 14]. They can be differentiated from the small granules by the lack of a less-electron dense outer matrix.

Table 1. – Distinct cytoplasmic structures of the human eosinophil leucocyte

<table>
<thead>
<tr>
<th>Spherical cytoplasmic structure</th>
<th>Appearance during differentiation</th>
<th>Constituents/function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary granule</td>
<td>Immature eosinophil leucocyte (promyelocyte)</td>
<td>Lysophosphatase</td>
</tr>
<tr>
<td>Secondary granule (&quot;specific&quot; granule)</td>
<td>Mature eosinophil leucocyte</td>
<td>Basic proteins (MBP, ECP, EPX), hydrolases (collagenase, β-glucuronidase) eosinophil peroxidase</td>
</tr>
<tr>
<td>Small granule</td>
<td>Mature eosinophil leucocyte</td>
<td>Arylsulphatase B, acid phosphatase</td>
</tr>
<tr>
<td>Microgranules (vesiculotubular-structures)</td>
<td>Mature eosinophil leucocyte</td>
<td>Transport system</td>
</tr>
<tr>
<td>Lipid bodies</td>
<td>Mature activated eosinophil leucocyte</td>
<td>Storage of unsaturated fatty acids</td>
</tr>
</tbody>
</table>

MBP: major basic protein; ECP: eosinophil cationic protein; EPX: eosinophil protein X.
Other prominent organelles in eosinophils are lipid bodies [15], which have also been identified in a wide variety of mammalian cells. Lipid bodies are round osmophilic, non-membrane-bound structures and often larger than specific granules. Their numbers increase during activation of eosinophils, and they appear to serve as intracellular sites of arachidonic acid storage and metabolism [15, 16], though their full function has not yet been delineated. Eosinophils also contain ubiquitous organelles, such as the rough endoplasmatic reticulum, free ribosomes, a small Golgi apparatus and mitochondria. The latter two seem to become more prominent in activated cells [17] (fig. 2).

Membrane receptors and surface markers

In recent years, a number of cell surface receptors and proteins have been identified on human eosinophils, which can be divided into binding sites for immunoglobulins, lipid mediators, complement proteins, cytokines and adhesion molecules [18]. A summary of the receptors currently recognized is given in figure 3.

Eosinophil leucocytes share many antigens found on other circulating white blood cells, such as the class I human leucocyte antigen (HLA) and the common leucocyte antigen CD45 [19, 20]. Although a specific epitope has not yet been identified, eosinophils can be differentiated from other leucocytes because they lack certain antigenic determinants. It is of particular significance that native eosinophils do not express the immunoglobulin G (IgG) receptor FcγRIII (CD16), an observation which is currently used to distinguish between eosinophil and neutrophil populations [21]. In addition, unlike monocytes, which express FcγRI and FcγRII, and neutrophils, which express FcγRII and FcγRIII, eosinophils bear only the FcγRII receptor (CDw32) on their membrane surface [20, 22]. However, a recent report has demonstrated that eosinophils cultured in the presence of interferon-γ (IFN-γ) induce both FcγRI (CD64) and FcγRII (CD16) [23]. In addition, whereas freshly isolated blood eosinophils respond poorly when challenged with anti-IgG and secreted only eosinophil cationic protein (ECP) [24, 25], INF-γ-exposed eosinophils responded to anti-CD16 antibodies with a significant release of leukotriene C4 (LTC4) [23].

Immunoglobulin E (IgE) receptors on human eosinophils have been demonstrated by the rosette technique (using IgE myeloma protein bound to red cells), as well as by radioligand studies using 125I-labelled IgE [26–28]. This receptor appears to be similar, but not completely identical, to the FcεRII (CD23) low-affinity receptors on lymphocytes, monocytes and other cells [28], since the CD23 antibody does not bind to the cells. There appear to be increased numbers of FcεRII receptors on hypodense and normodense eosinophils from patients with eosinophilia, when compared to eosinophils from normal subjects [29]. Functionally, binding of IgE leads to the release of eosinophil peroxidase (EPO) [30] and

![Fig. 3. Schematic representation of surface antigens identified on eosinophils. Some of the antigens are upregulated (+), downregulated (−) or induced (∗) following recruitment from the circulation into tissue. The existence of an IgE receptor on eosinophils is still a matter of ongoing debate (?)].

generation of platelet activating factor (PAF) [24], but fails to secrete ECP.

The same group [30] reported that human and animal eosinophils bind monomeric or secretory immunoglobulin A (IgA), though with a relatively low affinity. Only recently, the expression of FcœR on eosinophils was confirmed using immunofluorescence analysis of blood eosinophils from normal and allergic individuals [31]. Although neutrophils expressed much higher levels of FcœR than eosinophils in normal blood, eosinophils obtained from allergic subjects displayed enhanced FcœR expression, whereas neutrophils did not. Challenge of eosinophils with IgA and particularly the IgA-dimer causes significant degranulation, including the release of EPO [25, 30] and ECP [32].

Normal blood eosinophils do not express immunoglobulin M (IgM) receptors [29, 33, 34], although IgM binding to eosinophils can be induced by culturing them in the presence of cytokines [34]. The potential functional effects of IgM binding to eosinophils are as yet not known.

In addition to immunoglobulin receptors, a number of adhesion molecules have been detected on eosinophils, including the β1-integrin very late activation antigen (VLA) (CD29, CDw49), the β2-integrins CD11a, CD11b (CR3), CD11c and their common β-chain CD18, the immunoglobulin superfamily (intercellular adhesion molecule-1 (ICAM)-1, CD16, CD31, CD32, CD58), as well as selectins, the major histocompatibility antigen HLA-DR, CD4, CD69 and interleukin-2R (IL-2R) [19, 35–44]. Some of these surface antigens are expressed only on activated eosinophils (see below). The leucocyte integrins facilitate the intercellular adhesion of eosinophils to microvascular endothelium [45–47], and are essentially involved in the recruitment and migration of the cell into inflamed tissue (see below). They may also be involved in binding of other ligands, such as opsonized targets [48–52] and modifying other basic cellular functions.

Both eosinophil precursors and mature cells express the cell surface glycoprotein CD4 [53, 54], which has originally been known to be present on T-helper lymphocytes and on leucocytes of the monocyte/macrophage lineage. The CD4 binds human immunodeficiency virus-1 (HIV-1) gp 120 and can act as a signal transducer to elicit eosinophil migration [55]. Employing interleukin-5 (IL-5) stimulated bone marrow cultures, it has been shown that HIV-1 can infect eosinophil precursor cells in vitro [54], suggesting that eosinophils may serve as a reservoir for the virus in vivo.

Receptors for the complement factors C1q, C3a, C3b/C4b (CR1), iC3b (CR3), C4d and C5a have also been identified on human eosinophils [19, 56–59]. However, eosinophils have far fewer of these receptors than neutrophils and both the structure and affinity of the C5a receptor differs from that of the C5a receptor on neutrophils [60]. The proportion of complement bearing eosinophils ranges from 30% in normal individuals to 40% in patients with parasitic infestations [33]. The chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (fMLP) enhances the number of C3b receptors [61]. In addition, ECF-A, histamine and its major catabolite, imidazole acetic acid, have been reported to increase the expression of C3b and C4 receptors on human eosinophils, whereas C3d and IgG (Fcγ) receptors remained unaffected [56].

As mentioned above, eosinophils express receptors for iC3b (CR3), a degradation product of C3b. Functionally, CR3 appears to be similar to the receptor for C3b, causing binding of particles coated with iC3b to a phagocytic cell surface but failing to trigger ingestion in the absence of a second signal [62].

Eosinophils express a number of receptors for lipid mediators, including prostaglandin E (PGE), leukotriene B4 (LTB4), and PAF. PAF represents one of the most potent naturally occurring stimuli for eosinophils, inducing not only chemokinesis and chemotaxis but also release of granular proteins, production of oxygen radicals and synthesis of lipid mediators such as LTC4, thromboxane A2 (TXA2), and prostaglandin D2 (PGD2) [63, 64]. Its effect on eosinophils can be substantially augmented after exposure of eosinophils to certain cytokines, such as interleukin-5 (IL-5) or GM-CSF [4, 65–70].

Both functional and binding studies suggest the presence of two binding sites for PAF on eosinophils with an apparent KD of 0.33 nM and 11.5 nM, respectively [71]. Occupation of the high affinity PAF-RI receptor correlates with several functional responses, including degranulation and prostanoid release. In contrast, occupancy of the PAF-RII receptor is associated solely with the generation of O2·− radicals. The PAF receptor subsets utilize different signal transduction pathways with distinct intracellular second messengers and enzymes, which may account for their functional differences [71–73]. It is not clear, as yet, whether the two PAF receptors identified on eosinophils represent distinct binding sites, or whether they reflect two different affinity states of the same receptor.

Finally, several binding sites for cytokines have been detected on eosinophils, including the receptors for IL-3, IL-5, and GM-CSF [74–78]. Interestingly, these receptors show mutual cross-inhibition on eosinophils but not on neutrophils [74, 75], which may be due to a limited expression of the common β-chain by eosinophils [79].

Cellular constituents and products

Preformed granular constituents

The eosinophil constitutes a number of enzymes and highly biologically active preformed basic proteins which are stored in their cytoplasmic granules (table 2). The electron-dense crystalloid core consists of major basic protein, and the surrounding matrix is composed of a number of basic proteins and various hydrolytic enzymes.

Four different cationic proteins are recognized in the specific eosinophil granules [80]: the major basic protein (MBP), ECP, eosinophil-derived neurotoxin or protein X (EDN or EPX), and eosinophil peroxidase (EPO). Each of these proteins have been cloned and their biochemical and functional properties are well-established [81–88]. The most abundant cationic eosinophil granule protein is MBP. It is an arginine residue rich 14 kDa protein [82, 83, 85] devoid of any enzymatic activity, but highly toxic to helminthic parasites, tumour cells, and other mammalian cells and tissues. As will be
outlined below, MBP has toxic properties independent of oxygen metabolism. Like MBP, the "eosinophil cationic protein" (ECP) is a highly cationic protein, with toxic effects for helminthic and host cells. In addition, it shows a bactericidal activity [89]. ECP shares sequence similarities with the eosinophil-derived neurotoxin (EDN or EPX), and both polypeptides are of approximately the same size, ranging between 18–21 kDa [81, 84, 90, 91]. Both ECP and EDN induce cerebellar dysfunction, when injected intracerebrally into rabbits [80, 91, 92], as first described by Gordon [93] in 1930 (Gordon-phenomenon). In addition, these proteins have both partial sequence identity with pancreatic ribonuclease and possess a ribonuclease catalytic activity. Whilst EDN is about 100 times more potent as a ribonuclease than ECP [81, 91], it is devoid of any cytotoxic activity against parasites and host cells.

The fourth basic protein is the eosinophil peroxidase, which consist of two polypeptides of about 15 and 55 kDa [87, 88, 94, 95]. It is characterized by an overall negative charge but its major function comprises the formation of hypohalous acid in the presence of hydrogen peroxide and halide ions (preferentially bromide). Structurally and functionally EPO is distinct from the myeloperoxidase of neutrophils and monocytes and is toxic to helminthic and protozoan parasites, bacteria, tumour cells, and host cells [80, 96–99].

Table 2. – Preformed enzymatic and nonenzymatic proteins of the human eosinophil leucocyte

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Biological actions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonenzymatic proteins</strong></td>
<td></td>
</tr>
<tr>
<td>Major basic protein (MBP)</td>
<td>Helminthotoxic, cytotoxic for tumour cells and other mammalian cells, activates mast cells, neutralizes heparin</td>
</tr>
<tr>
<td>Eosinophil cationic protein (ECP)</td>
<td>Affects coagulation factors, helminthotoxic, neurotoxic, activates mast cells, cytotoxic for various mammalian cells, possesses RNase-activity</td>
</tr>
<tr>
<td>Eosinophil-protein X (EPX or EDN)</td>
<td>Strongly neurotoxic, helminthotoxic, inhibits lymphocyte cultures, RNase-activity</td>
</tr>
<tr>
<td><strong>Enzymatic proteins</strong></td>
<td></td>
</tr>
<tr>
<td>Eosinophil peroxidase (EPO)</td>
<td>Toxic for microorganisms, tumour cells and other mammalian cells, activates mast cells</td>
</tr>
<tr>
<td>Collagenase</td>
<td>Hydrolyses Type I and Type II collagen (connective tissue of the lung)</td>
</tr>
<tr>
<td>Arylsulphatase B</td>
<td>Hydrolyses proteoglycans and glycosaminoglycans</td>
</tr>
<tr>
<td>β-glucuronidase</td>
<td>Hydrolyses glycosides</td>
</tr>
</tbody>
</table>

EPX: eosinophil protein X; EDN: eosinophil-derived neurotoxic; RNase: ribonuclease.

De novo synthesized products

Eosinophils have, for some time, been known to synthesize oxygen radicals and lipid mediators (table 3). However, the eosinophil, like the neutrophil, has been

Table 3. – De novo generated mediators by the human eosinophil leucocyte

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Biological actions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
</tr>
<tr>
<td>PGE₂</td>
<td>Vasodilatation, mucus secretion, inhibition of inflammatory cells</td>
</tr>
<tr>
<td>PGD₂</td>
<td>Bronchoconstriction, pulmonary vasoconstriction, increase in vascular permeability, platelet aggregation</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>Bronchoconstriction, platelet aggregation</td>
</tr>
<tr>
<td>TxA₂</td>
<td>Bronchial and vascular constriction, platelet aggregation</td>
</tr>
<tr>
<td>LTC₄</td>
<td>Bronchial and vascular constriction, increase in vascular permeability</td>
</tr>
<tr>
<td>PAF</td>
<td>Bronchial and vascular constriction, bronchial oedema, platelet aggregation, mucus secretion, activation of neutrophils, mast cells and eosinophils</td>
</tr>
<tr>
<td>ECL</td>
<td>Eosinophil chemoattractant</td>
</tr>
<tr>
<td><strong>Oxygen metabolites</strong></td>
<td></td>
</tr>
<tr>
<td>Superoxide anion</td>
<td>Toxic for micro-organisms, tumour cells and other mammalian cells</td>
</tr>
<tr>
<td>Singlet oxygen</td>
<td>Toxic for micro-organisms, tumour cells and other mammalian cells</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td></td>
</tr>
<tr>
<td>IL-3</td>
<td>Priming and activation of inflammatory cells, granulocyte differentiation, enhances survival</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Priming and activation of inflammatory cells, granulocyte differentiation, enhances survival</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Activation of fibrocytes, Th2 cytokines inhibition</td>
</tr>
<tr>
<td><strong>Neuropeptides</strong></td>
<td></td>
</tr>
<tr>
<td>VIP</td>
<td>Bronchoconstriction, vasodilatation</td>
</tr>
<tr>
<td>Substance P</td>
<td>Bronchoconstriction, vascular oedema, vasodilatation, stimulates mucus secretion, degranulation of mast cells</td>
</tr>
</tbody>
</table>

PGE₂: prostaglandin E₂; PGD₂: prostaglandin D₂; PGF₂α: prostaglandin F₂α; TxA₂: thromboxane A₂; LTC₄: leukotriene C₄; PAF: platelet-activating factor; IL-3: interleukin-3; GM-CSF: granulocyte/macrophage colony-stimulating factor; TGF-β1: tumour growth factor-β1; VIP: vasoactive intestinal peptide; ECL: eosinophil chemotactic lipid.
assumed to be a short-lived, end-stage effector cell, with little or no capacity for protein synthesis. However, this assumption about the capabilities of mature eosinophils is probably not warranted. Blood eosinophils cultured in the presence of GM-CSF and murine 3T3 fibroblast [38], or eosinophils obtained from bronchoalveolar space during chronic inflammation [35, 37, 42], synthesize and express human leucocyte antigen-DR (HLA-DR), exemplifying the principal capacity of mature eosinophils to synthesize proteins. In addition, recent studies employing in situ hybridization suggest that eosinophils express the gene for GM-CSF, IL-3 and transforming growth factor-β1 (TGF-β1) [100–103]. Further, ionomycin-activated human eosinophils also gave specific immunocytochemical staining with α-human-cytokines [101], providing evidence for translation of cytokine messenger ribonucleic acid (mRNA). These data suggest that the mature eosinophil is not only capable of releasing de novo generated lipid mediators and oxygen radicals but can also synthesize new proteins.

**Oxygen radicals.** Eosinophils respond to stimulation with a respiratory burst generating toxic oxygen radicals, such as O$_2^\cdot$ [104–106], H$_2$O$_2$ [104,105,108–110], and probably I$_2$ as well as OH· [111, 112]. With most stimuli, the respiratory burst of eosinophils is greater than that of equivalent numbers of neutrophils [104, 105, 107, 109, 113]. A reduced nicotinamide adenine dinucleotide (NADPH) oxidase, similar to that present in neutrophils, appears to be involved [104, 106, 107]. NADPH oxidase activity was reported to be three to six times greater in eosinophils than in comparable neutrophil preparations [104], and eosinophils contain a cytochrome b$_{559}$ concentration that is twice that of neutrophils or monocytes [114].

**Lipid mediators.** Stimulation of human and animal eosinophils with various soluble and nonsoluble agents leads to the formation of various arachidonic acid-derived lipids, including LTC$_4$ [63, 69, 115–117], PGE [118, 119], prostaglandin F$_1$ (PGF$_1$) and TxA$_4$ [64, 119–123], PGD$_2$ and prostaglandin F$_2$α (PGF$_2$α) [64, 123]. In addition, eosinophils have been shown to synthesize large amounts of PAF [117, 124–126].

**Cytokines.** Recent work has demonstrated that eosinophils contain the genetic information for a number of cytokines. Various studies employing in situ hybridization suggest that eosinophils express the genes for GM-CSF, IL-3 and TGF-β1 as well as transforming growth factor-α (TGF-α) [100–103]. In addition, the cytokines could be detected immunocytochemically in human eosinophils when stained with monoclonal antibodies, providing evidence for translation of cytokine mRNA [100, 102]. Finally, IL-3 and GM-CSF were measured in ionomycin-stimulated supernatants [101]. The ability to release cytokines represents a new method by which eosinophils may contribute to inflammatory and immunological responses.

**Neuropeptides.** Neuropeptides represent a group of neurotransmitters which have originally been identified in neuronal tissue. However, recent studies have shown that some of these peptides, such as substance P (SP), vasoactive intestinal peptide (VIP) or somatostatin (SOM), are also released by immune cells and serve to modulate cytokine secretion by regulatory T-lymphocytes [127]. Eosinophils within the liver and intestinal granulomas of the mouse synthesize VIP and SP [128–130]. In addition, eosinophils have been demonstrated to release SP in vitro when exposed to histamine via activation of a H1 type receptor [129]. Furthermore, SP but not VIP has been shown to induce eosinophil degranulation, albeit through a toxic mechanism [131]. Hence, it appears that eosinophils also regulate local inflammation through the release of neuropeptides.

### Eosinophil heterogeneity

When examined under in vitro or in vivo conditions, eosinophils show differences in their morphological, phenotypical, physical and functional status (table 4). The significance of cellular heterogeneity is not yet known, but it may reflect a certain phase in the life cycle of the cell and facilitate adaptation of the eosinophil to the

<table>
<thead>
<tr>
<th>Table 4. – Characteristic cellular changes of the eosinophil following activation in vitro and in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellular changes</strong></td>
</tr>
<tr>
<td><strong>Morphology</strong></td>
</tr>
<tr>
<td>Dissolution of secondary granules</td>
</tr>
<tr>
<td>Decreased expression of surface antigens</td>
</tr>
<tr>
<td>Large empty vacuoles</td>
</tr>
<tr>
<td>Vesiculotubular structures</td>
</tr>
<tr>
<td><strong>Phenotype</strong></td>
</tr>
<tr>
<td>Increased expression of surface antigens</td>
</tr>
<tr>
<td>Induction of surface antigens</td>
</tr>
<tr>
<td>Decreased expression of surface antigens</td>
</tr>
<tr>
<td>CD69</td>
</tr>
<tr>
<td>CD4</td>
</tr>
<tr>
<td>ICAM-1 (CD54)</td>
</tr>
<tr>
<td><strong>Loss of surface antigens</strong></td>
</tr>
<tr>
<td><strong>Density</strong></td>
</tr>
<tr>
<td>Decrease in cellular density (hypodense cells)</td>
</tr>
<tr>
<td><strong>Function</strong></td>
</tr>
<tr>
<td>Desensitization</td>
</tr>
<tr>
<td>Hypersensitization (priming)</td>
</tr>
</tbody>
</table>

LFA-3: leucocyte function associated antigen-3; HLA-DR: human leucocyte antigen-DR; ICAM-1: intercellular adhesion molecule-1; VLA: very late activation antigen; PECAM-1: platelet endothelial cell adhesion molecule.
changing conditions or microenvironment during an acute inflammatory process. Cellular heterogeneity, however, is not specific for eosinophils, and has been also observed in alveolar macrophages [132] and neutrophils [133, 134].

**Morphological heterogeneity**

Eosinophils from patients with eosinophilia are morphologically distinct when compared to cells obtained from healthy subjects. The cells are characteristically hypodense and other changes include the appearance of cytoplasmic vacuoles, alteration in cell size and shape, increase in granule size, solubilization and loss of granular core and matrix proteins, together with an increase in vesiculotubular structures and smooth tubules and vesicles [8, 135]. Similar changes can be induced by exposure of eosinophils to cytokines or PAF in vitro [3, 4, 65, 66, 135, 136], suggesting that the morphological heterogeneity of eosinophils is caused by inflammatory mediators in vivo.

**Phenotypical heterogeneity**

It is an inherent property of cells to modify the expression of surface receptors, and eosinophils are no exception. Some receptors may be upregulated, as has been shown for the complement receptors, and adhesion molecules [19, 43, 137, 138]. Others are only synthesized and expressed in primed or activated eosinophils, such as Class II proteins of the major histocompatibility complex, CD4, ICAM-1 or IL-2 [37–42, 53, 139]. Others again, such as L-selectin are shed during eosinophil activation and transendothelial migration [35, 43].

An experimental approach in order to characterize such principal phenotypic changes of eosinophils undergoing activation is to compare bronchoalveolar lavage (BAL) and blood cells from different diseases [140]. As has recently been demonstrated, BAL eosinophils obtained from patients with atopic asthma and eosinophilic pneumonia showed an upregulation of the β2-integrins CD11b and CD11c, the aminopeptidase N (CD13), the glycoprotein CD24, the tyrosine phosphatase CD45, as well as the leucocyte function antigen-3 (CD58) by 51–222%. In contrast, the β2-integrins CD29 and CDw49 (VLA-4), the β2-integrin CD11a, the FcγRII (CDw32), the complement receptor CR1 (CD35) and other antigens (CD15, CD63, HLA-ABC) were downregulated by 30–73%. Furthermore, in both diseases the "intercellular adhesion molecule-1" (ICAM-1) was induced on BAL eosinophils, whilst L-selectin was shed by 94 and 98%, respectively. Interestingly, significant levels of the HLA-DR antigen were exclusively detected on BAL eosinophils from eosinophilic pneumonia; whereas, eosinophils obtained during the asthmatic late response from both BAL and peripheral blood did not express this class II major histocompatibility complex (MHC) antigen. Since expression of HLA-DR requires de novo synthesis, the reason why eosinophils 18 h post-antigen challenge do not express this antigen may simply be the limited time. The above data, however, suggest that tissue infiltrating eosinophils obtained during active disease undergo similar phenotypical changes, indicating a general mechanism of activation during recruitment into lung airways (see below).

The phenotypic changes observed in vivo can be imitated by appropriate treatment of native blood cells in vitro. For instance, blood eosinophils stimulated in vitro with IL-5, PAF or fMLP achieved similar levels of CD11b and VLA-4 expression as BAL eosinophils obtained 18 h post-challenge [20, 138, 141–144]. The stimuli also caused a decrease in L-selectin expression in eosinophils. Interestingly, whilst PAF and fMLP also induced comparable changes in neutrophils, IL-5 selectively affected the surface expression of adhesion molecules in eosinophils [141]. In contrast to blood cells, surface antigen expression on BAL eosinophils obtained 18 h post-challenge could not be further modified by either stimulus ex vivo [138]. Blood eosinophils cultured in vitro for 2 days in the presence of IFN-γ showed an upregulation of FcγRII (CDw32), and induced the expression of FcγRI (CD64) as well as a functionally active FcγRIII (CD16) receptor [23]. Taken together, these data suggest that surface receptor expression in eosinophils in vitro or in vivo follows a similar pattern, and indicates that eosinophils undergo activation during the recruitment process into the tissue.

**Density heterogeneity**

Eosinophils from healthy donors have the highest density of human peripheral blood leucocytes, and it is this property which forms the basis of the purification technique using density gradients. However, blood from patients with eosinophilia-associated diseases, such as the hyper-eosinophilic syndrome (HES), parasitic infection or allergy, contains a population of eosinophils which separate at a lower buoyant density. This population is termed "hypodense", as opposed to "normodense" eosinophils from normal individuals mentioned above [145]. Whilst normodense eosinophils represent approximately 90% of the cells found in normal subjects [146–148], peripheral blood eosinophils from patients with certain eosinophil-associated diseases, such as allergic rhinitis [149], asthma [146, 147, 150, 151], allergic bronchopulmonary aspergillosis and helminthic parasite infestation [2, 148], or an eosinophilic-myalgia-like syndrome [152], show an increased proportion of hypodense cells. In asthma, the mean percentage of hypodense blood eosinophils for patients with asthma in three studies varied between 35 and 65% [146, 147, 150]. In other active hyper-eosinophilic diseases, hypodense cells may account for up to 80% of the eosinophils [152].

In addition to blood cells, BAL eosinophils also constitute a high proportion of hypodense cells. This has been shown for eosinophils in the BAL fluid obtained from allergic asthmatics 48 h post-segmental allergen challenge [138, 153], chronic eosinophilic pneumonia [148, 154], as well as for other eosinophil-related disorders. In addition, a raised number of hypodense eosinophils have been found in pleural fluid recovered from patients with various lung diseases [148, 155].

The nature of this density heterogeneity and the mechanism(s) underlying the development of hypodense eosinophils...
is not yet known. In principle, hypodense eosinophils may represent: 1) a more mature, activated cell; 2) an immature cell; 3) a separate lineage of eosinophils; or 4) some combination of the above possibilities. However, the data so far available support the first hypothesis. Firstly, when exposed to PAF \textit{in vitro}, eosinophils undergo a time and concentration-dependent shift towards lower densities relative to the unstimulated control cells, thereby increasing the proportion of hypodense cells from 9% to 28% after 60 min and to 82% after 120 min [136]. The light-density eosinophils were found to be degranulated, contained less granules, showed an increase in cell volume, and upregulation of adhesion molecules [23, 136]. Similar morphological and phenotypic features were observed in the hypodense blood eosinophils of patients with allergy, eosinophilic pneumonia or parasitic infection [2, 18, 37, 145]. Secondly, incubation of normodense eosinophils, either with IL-3, IL-5 or GM-CSF, in the presence or absence of 3T3 fibroblasts, not only prolongs survival of eosinophils but also induces them to become hypodense (see reviews [65, 145]).

Taken together, these data clearly indicate that exposure of eosinophils to one or more mediators of inflammation induces a shift towards lower cell densities. Hence, a raised proportion of hypodense eosinophils may reflect activation of the cell and, in turn, an actively ongoing disease state. Since the cell density is determined by the ratio of cell weight to cell volume, the data so far available suggest that both loss of granular proteins and an increase in cell volume may contribute to the reduction of density in eosinophils [136].

Functional heterogeneity

The functional relevance of hypodense eosinophils is not yet understood and the data available appear somewhat conflicting. For instance, low density eosinophils from HES patients are cytotoxic to IgE-coated schistosomula, whilst normodense eosinophils are cytotoxic to IgG-coated schistosomula [156]. Secretion of eosinophil peroxidase by low density eosinophils from HES patients is caused by stimulation with antigen or anti-IgE, whilst normodense eosinophils from HES patients respond to anti-IgG [157]. In addition, eosinophils from patients with parasitic diseases show a slightly reduced density and an increased cytotoxicity towards IgG-coated chicken erythrocytes [158]. Low density eosinophils obtained \textit{in vitro} by incubation with GM-CSF are cytotoxic to IgG-coated schistosomula [65]. Furthermore, light density eosinophils from patients with HES generate and release greater amounts of leukotriene C4 in response to IgG or complement-coated targets and the monocyte-derived eosinophil-activating factor (EAF) compared to other stimuli [155, 159, 160]. Low density eosinophils from patients with HES show an increased deoxyglucose uptake and oxygen consumption and incorporation of PAF into the phospholipid pool than normodense cells [155, 161, 162] indicating a higher metabolic activity in light density eosinophils.

However, in contrast, some other studies indicate that hypodense eosinophils may also occur in a hyporeactive state. For instance, hypodense cells obtained from asthmatic subjects produced a lower amount of reactive oxygen metabolites in response to zymosan and phorbol myristate acetate (PMA), as assessed by nitro blue tetrazolium (NBT) reduction and chemiluminescence [4, 147]. In addition, low-density eosinophils obtained from asthmatic subjects produced only half the amount of LTC4 compared with normodense cells [115]. Furthermore, BAL, eosinophils from asthmatic subjects obtained 18 h post-allergen challenge showed a reduced capacity to generate prostanoids compared to blood eosinophils [163].

The reason for this discrepancy is not yet clear. Since an array of mediators are released during asthmatic inflammation [153, 164, 166], which may be responsible for both chemotactic attraction to the inflammatory focus and stimulation of cellular effector functions, the attenuated functional capacity of BAL eosinophils may be due to a cellular desensitization process, as has been previously described for eosinophils [72, 73, 167]. Interestingly, neutrophils recruited to the lung following segmental antigen provocation have also been found to be desensitized to LTB4, [168], suggesting that desensitization during tissue infiltration may be a necessary general process for accumulation at the spot with the highest level of chemoattractant. If this is true, one may hypothesize that hypodensity of eosinophils does not simply reflect cellular hyperreactivity. In contrast, the functional reactivity of eosinophils in relation to their density may be rather variable, depending on what factors the cell is exposed to in the microenvironment of the bronchial tissue, and could be controlled by inherent cellular feedback mechanisms which serve to protect the cell against overstimulation.

Activation and priming

An increasing number of substances with potential eosinophil-activating properties have been identified (table 5). Among them, calcimycin, also known as the Ca2+-ionophore A23187, a polyether antibiotic produced by \textit{Streptomyces chartreusensis}, and the tumour-promoting agent, PMA, represent valuable tools for evaluating the potential role of either Ca2+ or protein kinase C (PKC) and their interaction in eosinophil activation. However, since these agents are nonphysiological and cytotoxic at certain concentrations [146, 169, 170] they have no pathophysiological significance \textit{in vivo}. Other factors, such as the mast cell-derived acidic tetrapeptides, Val-Gly-Ser-Glu and Ala-Gly-Ser-Glu (eosinophil chemotactic factor of anaphylaxis (ECF-A)), which have been studied at several laboratories in recent years and which were considered to be selective eosinophil chemoattractants [171], are now known not only to be ineffective \textit{in vivo} [172] but also to show negligible activity \textit{in vitro} when compared to other stimuli [173, 174].

An increasing number of physiological agents that stimulate eosinophils have been identified, including lipid mediators such as LTB4 or PAF, cytokines, immunoglobulins and tachykinins such as substance P [65, 131, 175, 176], all of which may be best divided on the basis of their chemical structure and molecular weight into lipids, peptides and proteins.
of interleukin-2 in T-helper cells. Evidence to support this hypothesis in eosinophils can be drawn from results showing that the PAF receptor antagonist WEB 2086 partially inhibits thromboxane B\textsubscript{2} (TXB\textsubscript{2}) release from eosinophils stimulated with calcimycin [73, 119]. Hence, the release of PAF by eosinophils upon stimulation may result in an autocrine response amplification loop. However, PAF synthesized in eosinophils tends to remain cell-associated [125], and the possible role of PAF as an autocoid in eosinophil activation awaits further investigation.

A couple of studies indicate that PAF, in addition to its direct stimulatory effect, may also prime eosinophils [116, 199]. For instance, preincubation of human blood eosinophils with PAF for 30 min, significantly enhanced the calcimycin-induced LTC\textsubscript{4} production [116], as well as the respiratory burst induced by serum-treated zymosan [199], whereas LTB\textsubscript{4} failed to enhance the eosinophil mediator production [116]. Hence, taken together PAF would perfectly link the pathophysiological changes observed in asthma with the infiltration and activation of eosinophils. Unfortunately, however, PAF is not selective and acts on both neutrophils and eosinophils [186], suggesting that other mediators may also be operative in eosinophil recruitment (see below).

**Lipids.** Lipid mediators are derivatives of the membrane phospholipids and are de novo generated upon stimulation of the cell. Among them, leukotrienes and PAF have been implicated in the pathogenesis of various inflammatory diseases [175]. In asthma, for instance, PAF appears to represent one of the most effective mediators, capable of inducing bronchoconstriction [177, 178], microvascular leakage [179, 180], and airway hyperresponsiveness in animals [181] and humans [182]. Hence, PAF can mimic many of the changes occurring during the late asthmatic response. Furthermore, it has been demonstrated that PAF is a potent stimulus for several inflammatory cells, including eosinophils, and might be an important factor responsible for inflammatory cell infiltration in asthma [183–185]. In addition, there is evidence that eosinophil responsiveness to PAF may even increase during active disease [173, 186, 187]. PAF-elicted a spectrum of eosinophil functions, such as eosinophil chemotaxis, in vitro [173] and in vivo [188–191], eosinophil adhesion to endothelial cells [46, 192–194], eosinophil cytotoxicity against parasites [195], generation of superoxide anions [187, 196], release of granular enzymes and proteins [187, 196–198], and a shift in density from normodense to hypodense eosinophils [136] and several cyclooxygenase-derived arachidonic acid metabolites [64, 119, 121].

Not only is PAF a potent eosinophil-activating factor, but eosinophils are also the major source of this mediator [125]. As with other cells, this observation suggests that cells capable of producing a certain lipid mediator also respond to it. A major role of the lipids, therefore, may be to attract more effector cells to an inflammatory site through an amplification loop. In addition, PAF may function as an autocoid, modulating the eosinophil response induced by other agonists, such as C5a or fMLP. Such a function has been attributed to the mode of action of interleukin-2 in T-helper cells. Evidence to support

---

**Table 5.** Major currently-known eosinophil-activating factors subdivided according to their chemical nature

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipids</strong></td>
<td>Platelet-activating factor (PAF)</td>
</tr>
<tr>
<td></td>
<td>Leukotriene B\textsubscript{4}</td>
</tr>
<tr>
<td></td>
<td>8S, 15S-DiHETE</td>
</tr>
<tr>
<td></td>
<td>5, 15-DiHETE</td>
</tr>
<tr>
<td></td>
<td>Eosinophil chemotactic acid (ECL)</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>Granulocyte/macrophage-colony-stimulating factor (GM-CSF)</td>
</tr>
<tr>
<td></td>
<td>Interleukin-2</td>
</tr>
<tr>
<td></td>
<td>Interleukin-3</td>
</tr>
<tr>
<td></td>
<td>Interleukin-5</td>
</tr>
<tr>
<td></td>
<td>MIP-1\alpha</td>
</tr>
<tr>
<td></td>
<td>RANTES</td>
</tr>
<tr>
<td><strong>Complement factors</strong></td>
<td>C5a</td>
</tr>
<tr>
<td><strong>Immunoglobulins</strong></td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td></td>
<td>Immunoglobulin A (dimer)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>Phorbol myristate acetate (PMA)</td>
</tr>
<tr>
<td></td>
<td>Ca\textsuperscript{2+}-ionophore A23187 (calcimycin)</td>
</tr>
</tbody>
</table>

**DiHETE:** dihydroxyeicosatetraenoic acid; **MIP1\alpha:** macrophage inflammatory protein-1\alpha; **TPA:** tissue polypeptide antigen; **RANTES:** regulated upon activation in normal T-cells expressed and secreted.

**Peptides.** A number of peptides have been shown to stimulate eosinophils. SP induces eosinophil granular protein release, though presumably via a nonspecific mechanism [131]. Formyl-methionine-containing peptides such as fMLP are chemotactic for a number of cell types and show certain similarities to chemotactic factors produced by bacteria. Eosinophils express specific fMLP receptors [131, 200], and respond to the tripeptide with chemotaxis, degranulation, superoxide anion generation, and prostanoid production [73, 131, 200–202]. Although its effect is small compared to other stimuli, overnight culture of the cells in monocyte-conditioned medium substantially increases both receptor expression and eosinophil functional responses [200].

**Proteins.** Various classes of proteins, including immunoglobulins, complement factors and cytokines, possess chemotactic and stimulatory activities for eosinophils. Using rosetting techniques, IgG receptors had been demonstrated on eosinophils as early as 1969 [203]. More recent work has confirmed that mature eosinophils bear the FcyRII receptor [19, 23, 204], which is known to bind to multivalent ligands [205]. Thus, the eosinophil tends to bind to large surfaces coated with antibody, such as parasites. IgG receptor activation causes the generation of LTC\textsubscript{4}, respiratory burst and secretion of granular contents [170, 206]. In addition to FcyRII sites, receptors for IgE have been described on eosinophils [26, 27], and activation of this receptor promotes the release of granular proteins and PAF [24, 25]. However, the IgE receptor on eosinophils has not been fully characterized and appears not to be completely identical to FcεRII (CD23), the low-affinity IgE receptor on lymphocytes, monocytes and other cells [28]. Finally, monomeric IgA, and in particular secretory IgA (sIgA), have recently been shown to be potent eosinophil degranulating agents [32].
In addition, complement proteins, such as C3a and C5a, (assessed using opsonized parasites, zymosan or sepharose) have been implicated as eosinophil activators that facilitate chemotaxis (i.e. C5a) and binding of eosinophils to large surfaces, thereby inducing secretion of granular contents [2, 207–210]. In addition, C5a elaborates protein release, oxygen burst, and lipid prostanoid generation in human eosinophils [119, 196, 211, 212].

In recent years, an increasing number of cytokines relevant to eosinophils have been identified (table 6). For instance, the haematopoietic cytokines, IL-3, IL-5 and GM-CSF promote eosinophilopoiesis in vivo, prolong eosinophil survival and modulate eosinophil function in vitro (for review see [213]). Human peripheral-blood-derived normodense eosinophils cultured for 7 days with IL-3 [65] or GM-CSF [3, 66, 67, 214] generate increased amounts of LTC₄ and enhanced cytotoxicity toward schistosomula when challenged with calcimycin. Evaluation of the cell density revealed that exposure of eosinophils to the cytokines converted them to a hypodense phenotype. A similar effect on eosinophil releasability was observed with IL-5 [75, 215]. FUSAWA et al. [216] demonstrated that preincubation of normal eosinophils with IL-3, IL-5 or GM-CSF also enhanced the sIgA- and IgG-induced release of EDN. IL-5 was the most potent enhancer of immunoglobulin-mediated degranulation and its effect was apparent within 15 min. IL-5 also enhances eosinophil adhesion to endothelial cells [217] and induces eosinophil chemotaxis in vitro [218] and in vivo [219]. The particular significance of IL-5 for eosinophil-related inflammation is its selective action on eosinophils but not on neutrophils [220, 221].

In addition to their priming effect, cytokines elicit a direct stimulatory action on eosinophils. For instance, GM-CSF and IL-5 induced a small but significant release of EDN. Whilst IL-2 has no effect on degranulation, IL-2 together with the lymphocyte chemoattractant factor (LCF) has been shown to be by far the most potent eosinophil chemoattractant [40, 55]. Systemic or intrapleural administration of IL-2 induced a significant eosinophilia in the peripheral blood [222–224] and pleural fluid [225], respectively. The migratory response of eosinophils is mediated by the high affinity IL-2 receptor as evidenced by the molar potency of IL-2 as an eosinophil chemoattractant and by the ability of monoclonal antibodies to inhibit IL-2-induced chemotaxis [40].

LCF is a 56 kDa basic glycoprotein, that is elaborated by CD8+ human T-lymphocytes [226, 227]. LCF utilizes CD4 as its receptor and stimulates the migration of CD4+ mononuclear cells [226]. As has been mentioned above, CD4 expression is also detectable on eosinophils obtained from both normal donors and those with eosinophilia [53, 55], albeit at appreciably lower levels than found on T-helper cells. Since LCF does not stimulate migration of neutrophils, which lack CD4 expression, the mediator may contribute to the preferential tissue influx of both eosinophils and CD4+ lymphocytes in related disease.

Within the past 2 yrs more cytokines with chemotactic properties, which are members of a structurally-related low molecular weight platelet factor 4/intercrine/chemokine

| Table 6. – Cellular sources and biological actions of eosinophil regulatory cytokines |
|---------------------------------------|-----------------------------------------------|
| Cytokine | Cell sources | Biological action |
| GM-CSF | T-lymphocytes | Augments cell viability, adherence, degranulation, phagocytosis, superoxide anion production, leukotriene C₄ generation, and cytotoxicity |
| Mast cells | | |
| Macrophages | | Reduces cell density |
| Epithelial cells | | Stimulates proteoglycan synthesis |
| Eosinophils | | Uregulates CD11b, LFA-1 and PAF receptor expression |
| IL-1 | Many cell types | Augments adherence to endothelial cells and degranulation |
| IL-2 | T-lymphocytes | Eosinophil chemoattractant |
| IL-3 | T-lymphocytes | Augments viability, leukotriene generation and cytotoxicity |
| Mast cells | | Reduces cell density |
| Eosinophils | | Stimulates proteoglycan synthesis |
| IL-5 | T-lymphocytes | Augments viability, leukotriene generation and cytotoxicity |
| Mast cells | | Reduces cell density |
| Eosinophils | | Stimulates proteoglycans synthesis |
| LCF | CD8+ T-lymphocytes | Eosinophil chemoattractant |
| TNF-β | Various cell types | Augments adherence to endothelial cells and weak cellular cytotoxicity |
| IFN-γ | T-lymphocytes | Augments viability |
| | | Increases cytotoxicity (delayed acting) |
| IFN-α | Various cell types | Augments viability (weak) |
| MIP-1α | Lymphocytes | Eosinophil attractant |
| Eosinophils | | |
| RANTES | T-lymphocytes | Potent eosinophil chemoattractant |
| Platelets | | Induces ECP and oxygen radical release |

TNF: tumour-necrosis factor; IFN: interferon; ECP: eosinophil cationic protein; LCF: lymphocyte chemoattractant factor. For further abbreviations see legend to tables 3 and 5.
superfamily, have been identified. Although previously recognized as a neutrophil attractant, interleukin-8 (IL-8), a member of the C-X-C branch of the superfamily, has been shown to attract and activate eosinophils previously primed with IL-3, IL-5 or GM-CSF [228]. In addition, both macrophage inflammatory protein-1α (MIP-1α) and RANTES, two members of the C-C branch, are potent eosinophil attractants [229, 230] with a similar efficacy to the most potent chemotactic myeloid cell agonists, C5a and PAF. RANTES also induced secretion of ECP and oxygen radical release by human eosinophils [230]. Since RANTES also attracts CD45RO+ (memory) T-cells [231] but no neutrophils, local production of RANTES by activated T-cells and platelets may account, in part, for the characteristic inflammatory infiltrate observed in allergic disease.

Interaction with other cells

The eosinophil is an immunological cell and, as such, part of a highly complex system which requires a constant flow of mutual information among other participating cells. Multiple mechanism by which eosinophils may interact with other immunological cells have been suggested over the past years and will be outlined below (fig. 4).

Mast cells and basophils

Although of different origin, mast cells and basophils share a number of properties, including not only FcεRI expression and mediator content but also with respect to possible co-operation with eosinophils. For instance, a number of studies have demonstrated that MBP, ECP and EPO induce a non-cytolytic release of histamine both from human basophils [223, 232–238] and mast cells [239–241]. However, whilst basophils circulate in the blood and infiltrate tissue during late phase allergic reactions, mast cells are normally distributed throughout connective tissue [232, 233] and represent the first line inflammatory cell when exposed to allergen in atopic disease.

It has been shown that EPO binds to the negatively-charged mast cell granule to form a complex that retains toxic activity to bacteria [242] and tumour cells [243] when supplemented with H₂O₂ and a halide [242, 244]. Since EPO at high H₂O₂ concentrations and the EPO/H₂O₂/halide complex at lower H₂O₂ concentrations can initiate mast cell granule release [245], it is conceivable that mast cells and eosinophils may act synergistically to improve host defence. On the other hand, mast cell granules released during inflammation may contribute to the tissue irritation caused by other eosinophil proteins. It has been suggested that mast cells, which normally contain a small amount of granular peroxidase activity [244], may internalize exogenous EPO using a vesicular transport system and incorporate the peroxidase in their cytoplasmic granules [243, 246, 247]. Conversely, human mast cells and basophils may also counteract the toxic principles derived from eosinophils. For instance, a number of studies have demonstrated that human mast cells and basophils rapidly sequester toxic eosinophil proteins by endocytosis [248–250]. This observation may provide an explanation for the presence of MBP or EPO in basophils and mast cells [248, 251].
In addition, heparin and related anionic molecules, which bind histamine within mast cell and basophil granules, neutralize the toxic properties of MBP, as has been demonstrated for bronchial epithelial cells [252, 253] and tumour cells [254].

Finally, mast cell- and basophil-derived mediators, such as PAF or cytokines, may facilitate the function of eosinophils [233]. Since mast cells have been implicated in the immediate reaction in IgE-dependent hyperreactivity, the release of these mediators may contribute to the pathogenesis of the delayed infiltration of other inflammatory cells, such as eosinophils, in late allergic reaction.

**Neutrophils and macrophages**

Both neutrophils and macrophages may contribute to eosinophil-dominated inflammatory reaction via release of eicosanoids and reactive oxygen species and cytokines [255–258]. Toxic properties by intact neutrophils and macrophages are considerably increased when eosinophil-derived EPO is bound to the target cell surface, presumably due to a more sufficient utilization of H2O2 by the phagocytes [259]. This has been shown by the efficacy of macrophages in killing staphylococci [260], toxoplasma [261], and trypanosoma [262] as well as for macrophage-mediated cytolysis of neoplastic cells in vitro [263].

In addition, eosinophils may activate neutrophils through the release of granular proteins. A recent study suggests that MBP is capable of inducing oxygen radical and lysozyme release by neutrophils in a noncytotoxic concentration-dependent fashion, whereas other eosinophil-derived granular proteins had no effect [264]. A similar effect of eosinophil granular proteins has been proposed for macrophages [265].

Since both neutrophils and macrophages produce a number of mediators themselves, it is likely that they in turn are also capable of modulating eosinophil function. For instance, in vitro studies have demonstrated that supernatants of alveolar macrophages obtained from active chronic eosinophilic pneumonia are able to stimulate eosinophil chemiluminescence [148].

**Endothelial cells**

Since endothelial cells are located strategically at the interface between circulating blood cells and tissue, interaction with eosinophils are of particular interest with respect to transmigration from the circulation into the tissue. The significance of endothelial cells for eosinophils relates to their ability to release cytokines [266–268], PAF [269, 270], the co-operative synthesis of other lipid mediators [271], and the expression of adhesion molecules (for review see [272]). At the same time, endothelial cells represent a target for the action of these mediators [273].

Although the mechanisms facilitating transmigration of leucocytes are not yet completely understood, increasing evidence suggests that adhesion molecules expressed both on native and activated endothelial cells, as well as leucocytes, play a crucial role in orchestrating this process. For eosinophils, a number of adhesion molecules of different families, including leucocyte integrins, the selectins, members of the immunoglobulin family, and certain carbohydrates, have been demonstrated to mediate the interaction with vascular endothelial cells.

The endothelial cell expresses ICAM-1, ICAM-2, E-selectin (originally called endothelial-leucocyte adhesion molecule-1 (ELAM-1)), P-selectin (also known as GMP-140) and vascular cell adhesion molecule-1 (VCAM-1) [274]. ICAM-1, ICAM-2 and VCAM are glycoproteins belonging to the immunoglobulin gene superfamily, while E-selectin and P-selectin are members of the selectin gene superfamily.

Eosinophils, like neutrophils, monocytes and lymphocytes, constitutively or upon activation express adhesion molecules (see also section on Membrane Receptors and Surface Markers), which correspond to those on endothelial cells. For instance, leucocyte function-associated antigen-1 (LFA-1) (CD11a/CD18) or Mac-1 (CD11b/CD18) on eosinophils can bind to ICAM-1 (CD54) and ICAM-2, respectively, on endothelial cells [275–278]. The significance of ICAM receptors could be demonstrated in a primate model of asthma, in which administration of monoclonal antibody to ICAM-1 attenuated arterial eosinophilia and hyperresponsiveness [47]. However, in vitro anti-CD18 antibodies only partially inhibited eosinophil adherence to the vascular endothelium induced by PAF [46]. Moreover, in the leucocyte deficiency syndrome, in which there are genetic deficiencies in CD18 and impairments in the abilities of neutrophils to emigrate from the vasculature into sites of inflammation, both lymphocytes and eosinophils can be found in inflammatory lesions [279], indicating that these two cell types facilitate mechanisms of adherence independent of CD18.

Among the non-CD18-dependent pathways, the interaction between E-selectin (ELAM-1) on activated endothelial cells and sialoglycoproteins on eosinophils may be of biological relevance [275–277]. In addition, eosinophils, like neutrophils and mononuclear cells, express L-selectin (also known as lectin cell adhesion molecule-1 (LEC.CAM-1), lectin adhesion molecule-1 (LECAM-1), leucocyte adhesion molecule-1 (LAM-1), Mel-14, Leu8 or TQ1) which binds to sialoglycoproteins (glycine cell adhesion molecule (GlyCAM)) on endothelial cells and which is involved in the initial phase of adherence and later shed from the cell surface [35, 276, 280]. However, as for CD18-dependent pathways, adhesive mechanisms involving selectins are not selective. For instance, administration of anti-E-selectin monoclonal antibody in the primate model also blocked neutrophil accumulation in acute antigen-elicted airway inflammation [281].

Recent data suggest that endothelial cells and eosinophils may interact via a more selective mechanism. Both normal and activated eosinophils, but not neutrophils, express VLA-4 [43, 144, 275–277, 282]. VLA-4 is a β1-integrin and binds to VCAM-1 as well as to fibronectin on endothelial cells [283]. Blockade of both CD18 and VLA-4 integrin-mediated pathways abolished eosinophil adherence to TNF-α activated endothelia cells [277], whereas a combination of antibodies towards ICAM-1, VCAM-1
and E-selectin caused only partial inhibition [276, 277]. Since VCAM-1 also facilitates the adherence of lymphocytes and monocytes, but not neutrophils, the interaction between VCAM-1 and VLA-4 may represent an important specific mechanism for the concomitant recruitment of eosinophils and mononuclear leucocytes, into sites of allergic or other immunological reactions.

This conclusion was further supported by findings which demonstrated that VCAM-1 expression on endothelial cells is increased by IL-4, either alone [144] or in concert with TNF or IL-5 [284–286]. In addition, IL-4, which is also involved in the regulation of IgE synthesis, induced a VLA-4/VCAM-1-dependent adherence of eosinophils and basophils to endothelium, but not neutrophils [144]. In limited studies, augmented expression of endothelial VCAM-1, E-selectin, and ICAM-1 have been found in tissue sites of late-phase reactions [278, 286], and other tissue eosinophilia-related reactions [287, 288].

**Epithelial cells**

Eosinophils adhere to and transmigrate through epithelial barriers in asthma to reach the bronchoalveolar space [289, 290]. In addition to its function as a surface protector and cover, epithelial tissue represents a metabolically active secreting tissue, which may actively interact with inflammatory cells, such as eosinophils. Human airway epithelial cells produce GM-CSF [291–293] as well as PAF, PGE \(_2\), PGF \(_{2\alpha}\) [294] and 15-lipoxygenase pathway-derived eicosanoids [295], which may influence eosinophil function after arrival in the bronchoalveolar space. This function, however, may itself represent a target for anti-inflammatory drugs, such as corticosteroids [296]. Furthermore, as with vascular endothelial cells, airway epithelium expresses adhesion molecules, such as ICAM-1 [297, 298], and adherence of eosinophils to respiratory epithelium can be partially blocked by monoclonal antibody towards ICAM-1 [47].

**Platelets**

Since the discovery that platelets bear IgE receptors and can be activated during IgE-dependent events [299], the platelet is regarded as another accessory cell involved in allergic disease. Platelets can undergo chemotaxis, and phagocytosis and are able to release a number of pro-inflammatory mediators [300]. Data demonstrating the role of platelets derive from studies which show that, in a rabbit model, platelet depletion using a selective anti-platelet antiserum prevented antigen-induced bronchospasm [300, 301]. In addition, in guinea-pig, depletion of platelets virtually ablated bronchial hyperreactivity and eosinophil infiltration following exposure to PAF [302], indicating that platelets may be involved in eosinophil recruitment. Two potent eosinophil chemoattractants, PAF [173] and RANTES [229], are produced by platelets and may be involved in platelet-dependent eosinophil migration. Further evidence for the role of platelets in allergic inflammation stems from experiments which show that levels of granular platelet products, such as platelet factor-4 (PF-4) and β-thromboglobulin (β-TG), are raised in asthmatics after antigen challenge and correlated with elevations of eosinophil granule products [303, 304]. On the other hand, eosinophil products, such as MBP and EPO, have been shown to activate platelets [305].

**Lymphocytes**

Eosinophils and lymphocytes are recruited concomitantly into inflammatory sites [290, 306–309], suggesting a link between both cell types. The role of lymphocytes in relation to eosinophils is mainly determined by their production and release of cytokines. Lymphocytes are the source of the two most potent eosinophil chemoattractants known to date, LCF [226] and IL-2 [40, 309]. In addition, cytokines of the Th2 subtype, which is predominantly found in eosinophil-related conditions [310, 311], including IL-3, IL-5, and GM-CSF, probably promote eosinophilopoiesis, eosinophil recruitment, prolong eosinophil survival, and enhance eosinophil function [4, 18, 57, 215, 216, 220, 221, 228]. In addition, the products of recruited lymphocytes are likely to affect the function and subsequent recruitment of additional eosinophils and lymphocytes. For instance, IL-4 elaborated by Th2 cells effectively promotes VCAM-1 expression on endothelial cells [284, 285], which would further facilitate both recruitment and activation of eosinophils and lymphocytes (see above).

**Fibroblasts**

Several studies have demonstrated that the function, survival and physical properties of eosinophils are influenced *ex vivo* by incubation with monolayers of murine fibroblasts supplemented with certain cytokines [65]. These conditions not only promoted the survival of eosinophils, but also caused eosinophils to become hypodense and augmented eosinophil effector functions, such as lipid mediator secretion or antibody-dependent cytotoxicity of helminthic larvae. More recently, it has been shown that human lung-derived fibroblasts alone can promote eosinophil survival *in vitro* and that GM-CSF elaborated by these cells may be responsible for this effect [312].

On the other hand, since tissue fibrosis is associated with certain hyper eosinophilic syndromes, it had been argued that eosinophils may produce factors which induce and maintain fibrogenesis. This view is supported by findings which demonstrate that extracts obtained from human and guinea-pig eosinophils release mitogens for fibroblasts [313], and stimulate fibroblast deoxyribonucleic acid (DNA) synthesis [314].

**Recruitment of eosinophils**

**General mechanisms**

Adhesion of circulating leucocytes to vascular endothelium is a crucial process for effective host defence against infection and injury. In order to accumulate in tissues,
Circulating leucocytes must adhere to the endothelium lining, penetrate the vessel wall, and migrate to the site of tissue irritation. Acute bronchopulmonary inflammation involves a myriad of cellular and humoral mechanisms, which promote increases in vascular permeability, changes in blood flow, mobilization, accumulation and activation of leucocytes, all of which may have some bearing for eosinophils during emigration into tissue. On a cellular level, eosinophil recruitment involves specific and nonspecific adhesive interactions with vascular endothelial cells, penetration of the blood vessel wall, chemotaxis and accumulation at the site of allergic inflammation.

In recent years, several studies have led to a detailed insight into the possible mechanisms underlying eosinophil recruitment from the circulation into the tissue (fig. 5). This process can be divided arbitrarily into a sequence of steps:

1. **Random Contact**
   - Capillary endothelial cell
   - Eosinophil leukocyte
   - Flow direction

2. **Early Nonspecific Adhesion**
   - Diffusing mediators from the inflammatory focus
   - E-selectin
   - L-selectin

3. **Rolling**
   - Mediators

4. **Late Nonspecific Adhesion (Sticking)**
   - Activated endothelial cell
   - ICAM-1, ICAM-2, ICAM-1 PAF-R1, LFA-1 (CD11a/CD18), PAF-R1, Mac-1 (CD11b/CD18), Activated eosinophil

5. **Adhesion Molecule Expression**
   - Cytokines
   - PAF

6. **Activation**
   - Activated endothelium
   - Mediators
   - Activated eosinophils

7. **More Selective Eosinophil Adhesion and Attraction**
   - IL-4
   - IL-2
   - IL-5
   - Endothelial cell
   - VCAM-1, IL-2, IL-5, LCF, VCAM-1, VLA-4

8. **Diapeadesis and Chemotaxis**
   - Adventitia

**Fig. 5.** – Proposed sequence of eosinophil recruitment following immunological activation. IL: interleukin; VCAM-1: vascular cell adhesion molecule; VLA-4: very late activation antigen; LCF: lymphocyte chemoattractant antigen; ICAM: intercellular adhesion molecule; LFA: leucocyte function associated antigen; CD: cluster differentiation.
of six different, though partly overlapping, stages (fig. 5a-h):
1. Eosinophil emigration may be initiated by random contact of the cell with endothelium, which is most likely to occur in capillary vessels due to their small lumen (fig. 5a).
2. When in the vicinity of an inflammatory site, L-selectin receptors on naive nonactivated eosinophils may interact with locally-activated capillary endothelial cells bearing certain carbohydrate counter structures (possibly E-selectin) [315–317] (fig. 5b). Hence, L-selectin may function as an anchor, mediating an initial reversible adhesion. As a result of this early interaction between eosinophils and endothelial cells and the shear forces due to blood flow, the cells role or skid along the endothelial lining (rolling), thereby slowing the cellular flow possibly to provide the time required for cellular activation by locally-released mediators (fig. 5c).
3. Cytokines diffusing from the inflammatory site and through to the endothelial barrier, as well as cytokines and lipid mediators released by perivascular lymphocytes and macrophages and endothelial cells themselves, may now activate the eosinophil, resulting in both an increase in affinity and expression of adhesion molecules on the eosinophil surface (fig. 5d and e). Among the adhesion receptors involved are the β1-integrin VLA-4 (CDw49d/CD29), which binds to VCAM-1 on endothelial cells, and β2-integrin receptors LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18), and p150.95 (CD11c/CD18), which bind to ICAM-1. These and perhaps other events, in combination with endothelial activation and enhancement or de novo expression of adhesion molecules, are followed by a firm nonspecific (fig. 5f) and a more specific (fig. 5g) eosinophil-endothelial adhesion ("sticking").
4. Chemoattractants (IL-2, IL-5, RANTES, PAF) diffusing from the vicinity of the inflammatory site and through to the endothelial barrier, as well as cytokines and lipid mediators released by perivascular lymphocytes and macrophages and endothelial cells themselves, may now activate the eosinophil, resulting in both an increase in affinity and expression of adhesion molecules on the eosinophil surface (fig. 5d and e). Among the adhesion receptors involved are the β1-integrin VLA-4 (CDw49d/CD29), which binds to VCAM-1 on endothelial cells, and β2-integrin receptors LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18), and p150.95 (CD11c/CD18), which bind to ICAM-1. These and perhaps other events, in combination with endothelial activation and enhancement or de novo expression of adhesion molecules, are followed by a firm nonspecific (fig. 5f) and a more specific (fig. 5g) eosinophil-endothelial adhesion ("sticking").
5. The transmigrated cell now moves along a chemotactic gradient through the inflammatory microenvironment towards the inflammatory focus. In this phase, direct migration may be guided not only by increasing concentrations of chemotactic factors but also through adhesive interactions between eosinophil integrins and tissue extracellular proteins (for review see [272]).
6. As the eosinophil approaches the inflammatory focus, the cell is likely to get exposed to a mixture of increasing concentrations of activating lipid mediators and locally synthesized cytokines, lipid mediators, and neuropeptides. The spectrum and ratio of secreted mediators may determine the degree of eosinophil activation. Since some of the mediators at low concentrations preferentially prime, and at higher concentrations activate, eosinophil effector functioning [72, 320, 321], it is likely that, during the final approach to the inflammatory focus, the cells increasingly perform their cytotoxic function via release of basic proteins and generation of reactive oxygen species (fig. 6). At the same time, upon arrival at the inflammatory site, i.e. in the airway mucosa, the eosinophil may become immobilized and hyporeactive as a result of cell desensitization due to chemotactic factors, such as PAF [163–168, 318].
7. In the final stage, continued exposure of tissue or intraluminal eosinophils to cytokines released during ongoing inflammation by nearby tissue-dwelling cells...
[138, 163], endobronchial leucocytes or bronchial epithelial cells, however, may enhance survival and reprime the cell, enabling the eosinophil to regain its proinflammatory properties (paper submitted), namely the release of granule proteins, lipid mediators and reactive oxygen species.

More selective mechanisms

Since β₃-integrins are expressed on all leucocyte subsets, it is unlikely that they account for the characteristic inflammatory tissue infiltration observed in eosinophil-related disease. Therefore, in view of possible therapeutic approaches for these diseases, it is of great interest to understand the relative selective mechanism of eosinophil recruitment from the vasculature. To date, two principal mechanisms appear to be operative. Firstly, as outlined above (section on Membrane Receptor), the β₃-integrin VLA-4 (CD49d/CD29) has only been detected on eosinophils, basophils, lymphocytes, monocytes and dendritic cells but not on neutrophils [276, 278, 282–284], suggesting that interaction between VLA-4/VCAM-1 may be involved in more selective eosinophil and basophil recruitment. In addition, it has been shown that IL-4 induces VCAM-1 expression on endothelium, leading to the selective adhesion of human eosinophils and basophils [144, 213]. Hence, VLA-4 expression on eosinophils and binding to endothelial VCAM-1 may account for at least one mechanism by which eosinophils and basophils selectively migrate into inflamed tissue (fig. 5g).

A second possibility for the selective recruitment of eosinophils could be secretion of specific chemoattractants. Possible candidates mediating this mechanism are IL-2, IL-5 and LCF. Whilst IL-5 is only active on eosinophils, IL-2 and LCF also cause concomitant recruitment of CD4+ lymphocytes [322]. However, it is likely that other, as yet unknown, mechanism may be involved.

In view of this rather complex framework of interactions which appear to operate in vivo, one may ask whether it is absolutely necessary to hypothesize an absolutely specific chemoattractant or adhesion molecule for every single cell type. Selective recruitment of eosinophils can be sufficiently explained by the combination of several relatively selective recruitment events generating the conditions leading to selective eosinophil tissue infiltration.

Acknowledgement: The authors gratefully acknowledge A. Dewar and G. Zack-Kapp for preparing and providing the transmission and scanning electron micrograph figures.

References

1. Clutterbuck EJ, Hirst EMA, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and GM-CSF. Blood 1989; 73: 1504–1512.


55. Anwar ARE, Kay AB. Membrane receptors for IgG and complement (C4, C3b and C3d) on human eosinophils


146. Winquist I, Olafsson WT, Persson AM, Hallberg T. Altered density, metabolism, and surface receptors of
PULMONARY IMMUNE CELLS: EOSINOPHIL LEUCOCYTE

539

eosinophils in eosinophilia. *Immunology* 1982; 47: 531–539.


203. Henson PM. The adherence of leukocytes and platelets induced by fixed IgG or complement. *Immunology* 1969; 16: 107–121.


229. Kameyoshi Y, Dörschner A, Mallet AI, Christophers E, Schröder J. Cytokine RANTES released by thrombin-


266. Lamas AM, Marcotte GV, Schleimer RP. Human endothelial cells prolong eosinophil survival. Regulation


