Review Article

Eosinophils in the 1990s: New perspectives on their role in health and disease

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Introduction

Eosinophils have traditionally been somewhat neglected over the years by clinicians and pathologists alike, being seen as a sort of poor man's neutrophil, appearing in a supportive role as part of 'acute inflammation' with an occasional lead part in exotic diseases such as Churg–Strauss syndrome or hypereosinophilic syndrome. This neglect is unwarranted, both because the eosinophil is one of the most striking of cells, readily identified in haematoxylin and eosin (H&E) stained sections with their bilobed nuclei and bright red granules, and because the eosinophil is closely associated with two of the most common and universal of diseases, asthma and parasitic infection. The characteristic appearance of the eosinophil resulted in its early identification by Ehrlich in 1879 and its association with asthma and allergic disease was soon recognized.¹ Curiosity about its role in asthma and other diseases has persisted ever since. In recent years there has been an explosion of interest in the eosinophil reflected in an exponential increase in the number of published papers and books devoted to the cell.^{2–5} This interest has been mainly the result of increasing evidence that the eosinophil may be responsible for much of the tissue damage seen in asthma and the hope that modulation of eosinophil function may be an effective therapy for the disease. The purpose of this paper is firstly to review recent findings about the biology of the eosinophil, emphasizing the features that are distinctive about the eosinophil compared to other leucocytes and especially neutrophils. Secondly, I will briefly summarize the possible role of the eosinophil in the diseases with which it is associated.

Morphology and ultrastructure

Eosinophils are non-dividing, bone marrow-derived, granule-containing cells. They are approx-

imately 8 µm in diameter. One of their most characteristic features are the membrane-bound specific granules of which there are about 20 per human eosinophil. These are spherical or ovoid, and contain a crystalline core surrounded by a less electron-dense matrix.⁶ The core is comprised of major basic protein (MBP) and the matrix contains the other three basic granule proteins, eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil-derived neurotoxin (EDN or EPX).⁷ These basic proteins stain avidly with dyes such as eosin from which the cell gained its name. Eosinophils also contain lipid bodies which are non-membrane-bound organelles and the principal store of arachidonic acid esterified into glycerophospholipids.⁸ Eosinophil primary granules are a third type of intracellular organelle which contain Charcot–Leyden crystal (CLC) protein. CLC protein is also found diffusely in the nucleus and cytoplasm in activated eosinophils. Primary granules are recognized by the absence of a core and are of variable size, being often larger than the specific granules. They make up approximately 5% of eosinophil granules.⁹ Tissue eosinophils also contain a number of small granules which stain intensely for acid phosphatase and aryl sulphatase.^{10,11}

Eosinophil production

Eosinophils like other leucocytes differentiate from stem cell precursors in the bone marrow. They then migrate into the peripheral blood where they circulate with a half life of about 18 hours before migrating into tissue. Eosinophils are primarily tissue-dwelling cells with about one blood eosinophil for every 100 tissue eosinophils.¹² Normal human adult bone marrow contains about 3% eosinophils of which a third are mature and two-thirds are myelocytic precursors. Eosinophilic myelocytes are large cells with a single-lobed nucleus, expanded Golgi and extensive dilated cisterns of rough endoplasmic reticulum. They

become identifiable when they develop the core containing specific granules which initially are interspersed with large numbers of homogenous dense granules.¹³

There is now substantial evidence that the massive increase in eosinophils associated with helminthic parasitic infection is T-cell dependent.^{14,15} Three T-cell-derived cytokines have been shown to promote eosinophil growth and differentiation, interleukin-3 (IL-3), IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF). Eosinophil and basophil colonies appear together in colony-forming assays.¹⁶ Mouse IL-5, but not human IL-5, is a growth factor for B cells. IL-5 is a disulphide-linked homodimeric glycoprotein with a molecular weight of 40–45 kDa.^{17,18} The dimers are aligned in a head to tail fashion and dimerization is essential for function.¹⁹ Culture of mouse bone marrow suggested that IL-5 was a late differentiation factor and could not support eosinophil growth from early precursors. These steps appeared to require other cytokines such as IL-3 and GM-CSF.²⁰ However, IL-5 transgenic mice had a marked peripheral blood and tissue eosinophilia with increased numbers of eosinophil precursors in their bone marrow.^{21,22} Despite a marked eosinophilia these mice had no obvious pathological defect. The observation that IL-5 alone was sufficient to generate an eosinophilia is consistent with the fact that increases in numbers of eosinophils are often seen without expansion of the other myeloid lineages. Both IL-3 and GM-CSF induce eosinophil production *in vitro* in human cord blood culture²³ and after *in vivo* administration, although the increase in the number of eosinophils was modest compared to other lineages.²⁴ In humans the genes for IL-3, IL-4, IL-5 and GM-CSF are clustered on the long arm of chromosome 5.²⁵ The receptors for IL-3, IL-5 and GM-CSF are structurally similar.²⁶ They consist of unique though homologous α chains, which bind with low affinity to their respective cytokines with a K_d in the region of 10 nmol/l. There is a common β chain which is non-covalently associated with the α chains at the cell surface and transforms the receptor into one of high affinity (K_d 150 picomoles (pm)). The β chain is required for signal transduction. Unlike the α chains of IL-3 and GM-CSF, the α chain of the IL-5R can bind IL-5 with relatively high affinity (K_d 250–590 pm).^{27,28}

In many conditions associated with increases in eosinophils, including asthma,²⁹ parasitic disease,³⁰ IL-2 therapy,³¹ hypereosinophilic syndrome (HES)³² and eosinophilia/myalgia syndrome,³³ evidence of increased IL-5 production has been obtained. IL-5 messenger RNA (mRNA) has been detected in eosinophilic Hodgkins disease.³⁴ Antibodies against IL-5 abolishes the eosinophilia in parasitized animals.³⁵ Although IL-5 has been

detected in mast cells and eosinophils,³⁶ it is likely that T-lymphocytes are the principal source of this cytokine. T cells, as well as being divided into subsets on the basis of their receptor phenotype, can be distinguished by their cytokine profile. T-helper type 1 (Th1) cells produce IL-2 and interferon- γ (IFN- γ), Th2 cells IL-4 and IL-5, whereas GM-CSF and IL-3 are elaborated by both cell types.³⁷ T-cells with a Th2 profile of cytokine production are found in allergic and eosinophilic parasitic disease. Eosinophilia in many diseases therefore appears to be due to a specific type of T-cell response to certain types of antigen. For example, allergens in allergic disease and parasitic antigens in helminthic infections. Drug-induced eosinophilia may be due to the drug acting as a hapten for a Th2 response.

Eosinophil heterogeneity

Peripheral blood eosinophils from normal individuals are relatively dense cells which separate out from other leucocytes in the lower bands of Percoll or Metrizamide discontinuous density gradients. For many years these differences were the basis for the standard method of purifying eosinophils. This has now been largely superseded by immunomagnetic selection based on differences in expression of the low-affinity (immunoglobulin receptor. (Fc) γ RIII, cluster of differentiation (CD)16) IgG receptor by eosinophils and neutrophils.³⁸ A proportion of eosinophils from individuals with a raised eosinophil count are less dense than eosinophils from normal subjects.³⁹ The mechanism for this heterogeneity is unclear. Hypodense eosinophils appear to be vacuolated and contain smaller-sized granules, although of equal numbers to normal density eosinophils.⁴⁰ It is generally considered that hypodense eosinophils represent an activated phenotype.⁴¹ Thus hypodense eosinophils have increased oxygen consumption,⁴² increased cytotoxicity towards helminths⁴³ and increased leukotrine C4 (LTC4) production.⁴⁴ They release less platelet-activating factor (PAF) after stimulation with IgG sepharose beads but this appears to be the result of increased acetyl hydrolyase activity.⁴⁵ Stimulation of eosinophils either in the short term with PAF or in long-term culture with cytokines results in a hypodense phenotype and enhanced effector function.⁴⁶ In contrast, hypodense eosinophils have a similar profile of leucocyte integrin and Fc γ receptor expression to normal density cells⁴⁷ and normal density cells from individuals with an eosinophilia are also primed. Nonetheless, the weight of evidence suggests that hypodensity represents a primed or partially activated phenotype.

Eosinophil receptors

Like all leucocytes, eosinophils express various membrane receptors through which they communicate with the extracellular environment. These include adhesion receptors, Fc receptors and receptors for the recognition of soluble mediators (Figure 1).

Adhesion receptors

A critical aspect of leucocyte function is migration from the vascular space into extracellular tissue. The initial step in this process is adherence to postcapillary venular endothelium. This is mediated by binding of adhesion receptors on the surface of leucocytes to their ligands or counterstructures on endothelium. Adhesion receptors are grouped into several gene superfamilies, and include the integrin superfamily, members of the immunoglobulin superfamily, and the selectins.^{48,49} Integrins bind to members of the immunoglobulin receptor family and selectins bind, via their lectin domain, to carbohydrate counterstructures that include the moiety sialyl Lewis X.⁵⁰ Transmigration through vascular endothelium is a staged process in which the leucocyte is first tethered to the endothelial cell by binding of a selectin receptor to its carbohydrate ligand. The binding affinity of this

interaction is relatively weak and the leucocyte rolls along the surface of the endothelium until it comes into contact with a priming stimulus such as a chemotactic mediator. This allows the leucocyte integrin receptor to bind to its corresponding immunoglobulin-like ligand. The resultant bond is much firmer than the selectin carbohydrate bond and results in the leucocyte flattening and trans-migrating between endothelial cells.⁵¹ Three events are therefore required for migration to occur: (1) engagement of a selectin and its receptor; (2) leucocyte activation; and (3) engagement of the integrin/immunoglobulin receptor bond. Having transmigrated through the endothelium, the leucocyte interacts with the extracellular matrix proteins through its integrin and other adhesion receptors. Adhesion receptors and their ligands potentially involved in eosinophil function are summarized in Table I. As with neutrophils, L-selectin is shed when eosinophils are stimulated and BAL eosinophils that have migrated into the airways express very little L-selectin.⁵²

One potential mechanism for preferential localization of eosinophils (as opposed to neutrophils) at inflammatory foci is a selective adhesion pathway. IL-5 and IL-3 increase eosinophil, but not neutrophil, adhesion to unstimulated human umbilical vein endothelial cells (HUVEC). Eosinophils but not neutrophils can utilize the very

Figure 1 Schematic representation of an eosinophil with its bilobed nucleus and specific granules illustrating the major eosinophil membrane receptors.

Table 1 Eosinophil adhesion receptors and their counterstructures

Eosinophil receptor	Endothelial receptor	Matrix protein
Integrin		
VLA-4 ($\alpha 4\beta 1$)	VCAM-1	Fibronectin
$\alpha 4\beta 7$	MadCAM-1	Fibronectin
VLA-6 ($\alpha 6\beta 1$)		Laminin
LFA-1	ICAM-1, ICAM-2	
Mac-1	ICAM-1	Fibrinogen
Immunoglobulin-like		
PECAM	PECAM	
Selectin		
L-selectin	GlyCam-1	
Carbohydrate		
P-selectin ligand	P-selectin	
E-selectin ligand	E-selectin	

late antigen (VLA)-4/vascular cell adhesion molecule (VCAM)-1 pathway.⁵⁴ VCAM-1 expression is selectively upregulated by IL-4⁵⁵ and IL-4 is generated at sites of allergic inflammation.⁵⁶ IL-4 enhances eosinophil transmigration through endothelium in a VLA-4/VCAM-1-dependent manner.⁵⁷ IL-4 transgenic mice have a tissue eosinophilia and manifest an inflammatory condition in the eye similar to allergic conjunctivitis.⁵⁸ Anti-VLA-4 mAb's inhibit eosinophil migration into tissue in guinea pigs.⁵⁹ In contrast, VCAM-1 expression in eosinophilic tissue, such as nasal biopsies, nasal polyps, and endobronchial biopsies from asthmatic individuals and in the skin of allergic individuals after allergen challenge, is very weak or non-existent despite strong expression of other endothelial adhesion molecules.^{60,61} The evidence that selective expression of adhesion molecules is responsible for eosinophil localization in tissues in humans is therefore at best contradictory.

Eosinophils adhere to a number of extracellular matrix proteins. For example, fibronectin binds to eosinophils through VLA-4, laminin through VLA-6 and hyaluronate through CD44. Adhesion to matrix proteins results in priming for increased LTC₄ and hydrogen peroxide release.^{62,63} Eosinophils also survive for prolonged periods when cultured on fibronectin, as a result of auto-crine stimulation of IL-3 and GM-CSF production.⁶⁴ This is a possible mechanism for prolonged survival of tissue eosinophils in both health and disease.

Immunoglobulin receptors

The eosinophil expresses receptors for IgG, IgA and IgD. The eosinophil also binds IgE and

eosinophils can undertake a number of IgE-dependent functions, including killing of schistosomes opsonized with specific IgE.⁶⁵ It was thought that the eosinophil IgE receptor was related to the low-affinity IgE receptor found on B-lymphocytes, platelets and macrophages – Fc ϵ RIII (CD23).⁶⁶ However, peripheral blood eosinophils inconsistently express messenger RNA (mRNA) for CD23 and do not stain with a panel of monoclonal antibodies (mAbs) directed against this receptor. Eosinophils express the IgE-binding protein Mac-2 but so do neutrophils that lack IgE-dependent functions.⁶⁷ The nature of eosinophil IgE binding therefore remains to be clarified. There are three receptors for IgG: the high-affinity receptor, Fc γ RI (CD64), and two low-affinity receptors, Fc γ RII (CDw32) and Fc γ RIII (CD16).⁶⁸ Only CD32 is constitutively expressed by eosinophils to any significant degree.⁴² A number of eosinophil functions are mediated via this receptor, including schistosomula killing, phagocytosis, the secretion of granule proteins, and the generation of newly formed, membrane-derived lipid mediators such as PAF and LTC₄. After stimulation for 2 days *in vitro* with IFN- γ , eosinophils express CD16 and CD64 as well as CD32.⁶⁹ The eosinophil also expresses IgA receptors that, when engaged by IgA-coated Sepharose beads, trigger substantial release of eosinophil granule proteins.⁷⁰

Other receptors

An interesting feature of the eosinophil is its ability to express receptors *de novo* after prolonged (> 48 hour) culture in a number of cytokines. For example, after culture in GM-CSF, eosinophils express human leucocyte antigen (HLA-DR) antigens and increased amounts of ICAM-1, which are associated with an *in vitro* capacity to present antigen to T cells.⁷¹ Peripheral blood eosinophils express the early activation antigen CD69 after cytokine stimulation *in vitro* as do bronchoalveolar lavage (BAL), eosinophils from patients with asthma and pulmonary eosinophilia.⁷² Eosinophils also respond to a number of soluble mediators such as f-MLP, C5a, C3a and RANTES,⁷³ as well as to lipid mediators such as PAF, by means of specific receptors, many of which belong to the rhodopsin family of seven transmembrane region G-protein-linked receptors.

Eosinophil chemotaxis

A number of eosinophil chemotaxins have been described, although few are both effective and specific. PAF and C5a are highly active on human eosinophils but are equally active on neutrophils.⁷⁴ LTB₄ and 5,15-diHETE act as guinea pig eosino-

phil and neutrophil chemoattractants.⁷⁵ IL-5; IL-3 and GM-CSF have variable and generally weak activity in the Boyden chamber assay, although they are active at very low concentrations.⁷⁶ They are only active on eosinophils from individuals with normal eosinophil counts. However, they can effectively prime eosinophils for enhanced chemotactic responsiveness to suboptimal concentrations of PAF and LTB₄, as well as enhancing the generally negligible activity that f-MLP and IL-8 have for unactivated eosinophils.⁷⁷ Recently, it has been reported that the C-C chemokine RANTES is an effective and selective (in the sense of having no activity for neutrophils) chemoattractant for eosinophils.⁷³ *In vivo* PAF injected into the skin caused the accumulation of eosinophils in atopic individuals, but neutrophils were prominent in non-atopic subjects.⁷⁸ Increased numbers of neutrophils, but not eosinophils, appeared in BAL fluid 4–6 hours after the inhalation of PAF in a group of eight normal subjects, three of whom were atopic.⁷⁹ A highly effective but non-specific mediator such as PAF could combine with a selective but weakly chemotactic agent such as IL-5 to promote the specific accumulation of eosinophils in allergic disease. Inhalation of LTE₄ produced eosinophil migration into the airways.⁸⁰ The mechanism of this effect is not clear.

Eosinophil mediators

Eosinophils have the capacity to secrete a number of potent mediators (Figure 2). These include basic proteins stored in eosinophil granules, lipid mediators newly formed after eosinophil activation, cytokines, various eosinophil proteases, and components of the oxygen burst, including superoxide and hydrogen peroxide.

Lipid mediators

Eosinophils generate an array of lipid mediators principally eicosanoids and PAF.⁸¹ Eosinophils can generate relatively large amounts (up to 70 ng/10⁶ cells) of the sulphidopeptide leukotriene, LTC₄, after stimulation with the calcium ionophore, but only negligible amounts of LTB₄.⁸² This is in contrast to neutrophils which can produce large amounts of LTB₄, but little, if any LTC₄. LTC₄ generation by human eosinophils was also observed after stimulation with both opsonized zymosan and via an FcγII-dependent mechanism using Sepharose beads coated with IgG.⁴⁴ Eosinophils can also generate substantial quantities of 15-HETE via 15-lipoxygenase. Eosinophils generated 25 and 2 ng/10⁶ cells of PAF after stimulation with calcium ionophore and IgG.

Figure 2 Schematic representation of eosinophil-derived mediators.

coated Sepharose beads, respectively.⁴⁵ Much of the PAF remained cell associated. Eosinophils can also generate mediators of the cyclooxygenase pathway, including prostaglandins E₁ and E₂ and thromboxane B₂ (TXB₂).

Eosinophil granule proteins

MBP has a molecular weight of 13.801 and a pI of 10.9. It contains 17 arginine residues, which accounts for its basicity. It is initially synthesized as an acidic proprotein, which may neutralize MBP's toxicity while it is stored in the eosinophil granule.⁸³ Purified MBP was shown to be cytotoxic for the schistosomula of *S. mansoni*, and adherence of eosinophils to IgG-coated schistosomula resulted in the secretion of MBP on to the integument of the larvae.⁸⁴ MBP at concentrations as low as 10 µg/ml has also been shown to be toxic for both guinea pig and human respiratory epithelial cells.⁸⁵ The inhalation of MBP, albeit at high concentrations (1 mg/ml), produced increased bronchial hyperresponsiveness in monkeys.⁸⁶ MBP and EPO were shown to be strong agonists for platelet activation as well as inducing the non-cytolytic activation of mast cells, basophils and neutrophils.⁸⁷ The mechanism of action of MBP is likely to be related to its hydrophobicity and strong negative charge.

EPO is a heme-containing protein composed of a 14,000 Da (light) and a 58,000 Da (heavy) subunit derived from the same strand of mRNA and subsequently cleaved. The cDNA also demonstrates the presence of a prosequence.⁸⁸ EPO shares a 68% amino-acid identity with human neutrophil myeloperoxidase as well as other peroxidase enzymes. EPO is toxic for parasites, respiratory epithelium, and pneumocytes, either alone, or (more potently) when combined with H₂O₂ and halide, the preferred ion *in vivo* being bromide.

ECP is an arginine-rich protein with a pI of 10.8 of 133 amino acids with a molecular mass of 15.6 kDa. ECP shows 66% amino-acid homology with EDN and 31% homology with human pancreatic ribonuclease.⁸⁹ It has low ribonuclease activity compared to EDN. It appears to be expressed only in eosinophils or eosinophilic cell lines. ECP is toxic for helminthic parasites, isolated myocardial cells, and guinea pig tracheal epithelium. ECP also inhibits lymphocyte proliferation *in vitro*. Both ECP and EDN produce neurotoxicity (the Gordon phenomenon) when injected into the cerebrospinal fluid of experimental animals. The secreted form of ECP differs structurally and antigenically from the stored form. This difference has been used to differentiate between resting eosinophils and activated eosinophils in which active secretion is occurring with the mAb EG1 recognizing the stored form and the mAb EG2, the activated state.⁹⁰

EDN, also called EPX, is a 16 kDa glycosylated protein possessing marked ribonuclease activity. Like ECP, it is a member of ribonuclease multigene family.⁹¹ EDN expression is not restricted to eosinophils, as it is found in mononuclear cells and possibly neutrophils. It is also probably secreted by the liver. It does not appear to be toxic to parasites or mammalian cells and its only known function, other than its ribonuclease activity, is the neurotoxicity exhibited in the Gordon phenomenon.

A major constituent of eosinophil is CLC protein which has been shown to be lysophospholipase. It constitutes up to 10% of eosinophil protein and is also found in large quantities in basophils, thus highlighting the similarities between these two cell types. Its function is unclear.⁹²

Cytokines

Eosinophils can synthesize an array of cytokines. Activated eosinophils have been shown to secrete significant amounts of transforming growth factor-α (TGF-α).⁹³ After stimulation with calcium ionophore, eosinophils can also generate GM-CSF and IL-3, which prolong eosinophil survival as well as IL-8.^{94–96} Eosinophils in allergic tissue expressed mRNA for IL-5 and eosinophils have been shown to generate significant quantities of TGFβ and IL-6.^{97–99} IL-1 has also been detected in human eosinophils.

Other mediators

The eosinophil contains a number of granule-stored enzymes whose role in eosinophil function is not clear (reviewed by Spry¹⁰⁰). They include acid phosphatase (large amounts of which have been isolated from eosinophils), collagenase, arylsulphatase B, histaminase, phospholipase D, catalase, non-specific esterases, vitamin B₁₂-binding proteins and glycosaminoglycans. Eosinophils can undergo a respiratory burst with release of superoxide ion and H₂O₂ in response to stimulation.

Eosinophil secretion and activation

A striking feature of eosinophil-rich inflammatory reactions is the marked deposition of granule proteins often in the presence of relatively small numbers of intact eosinophils. The mechanism of eosinophil secretion *in vivo* is still poorly understood. Eosinophils are cytotoxic for the larvae of helminthic parasites such as schistosomulae of *S. mansoni* but only when the larvae have been opsonized with either complement or immuno-

globulin suggesting that triggering of eosinophil secretion is dependent on perturbation of Fcγ or complement receptors, particularly Mac-1. Eosinophils preferentially secrete their mediators on to a large surface, a process described as frustrated phagocytosis. Opsonized zymosan interacts with eosinophils, triggering generation of hydrogen peroxide and PAF through Mac-1.¹⁰¹ The ability of eosinophils to secrete their mediators is markedly enhanced by priming with soluble mediators such as chemotactic factors and cytokines. Chemotactic agents can also elicit the direct secretion of both granule proteins and lipid mediators, although soluble mediators are generally ineffective secretagogues except with highly activated eosinophils or when used in conjunction with cytochalasin B, which inhibits cytoskeletal assembly.¹⁰² Differential secretion of granule proteins depending on the stimulus has been reported. Immunoglobulin G (IgG) complexes induced the secretion of ECP but not EPO, whereas IgE complexes induced secretion of EPO but not ECP.¹⁰³ However, secretion was low in both instances. Eosinophils release their granule components by exocytosis, with individual granules fusing with the plasma membrane. This process involves a guanosine 5'-triphosphate (GTP)-binding protein and is modulated by the intracellular calcium concentration.¹⁰⁴ Priming of eosinophils involves a rise in intracellular calcium and triggering of PI turnover. As is the case with degranulation, the signal transduction pathways involved in priming appear broadly similar to those described for mast cells and neutrophils.¹⁰⁵

Several cytokines have a marked effect on eosinophil function. IL-5, besides being a growth maturation factor for eosinophils, also selectively stimulates a number of mature human eosinophil functions, including prolonged survival, cytotoxicity toward helminth targets, and increased adhesion to vascular endothelium.¹⁰⁶ IL-3 and GM-CSF have similar though less selective activities. IFN-γ stimulates eosinophil cytotoxicity, prolongs eosinophil survival and results in expression of mRNA for GM-CSF. TNF-α stimulated eosinophil cytotoxicity toward endothelium.¹⁰⁷ IL-3, IL-5 and GM-CSF have both short-term priming effects on eosinophils, which are maximal within an hour and more long-term effects, which include increased receptor expression and depend on protein synthesis.

Eosinophilia

An isolated count of eosinophil numbers in the blood offers only a limited and sometimes

misleading picture of eosinophil involvement in a particular disease. The blood eosinophil count represents the balance between the rate of eosinophil migration from the bone marrow and entry into the tissues. Once in the tissue, eosinophils can survive for many days under the influence of locally generated cytokines. Their removal appears to be largely the result of programmed cell death (apoptosis) and subsequent phagocytosis of the senescent cell by macrophages.¹⁰⁸ Despite the usually modest peripheral blood eosinophil counts, there are large numbers of eosinophils and their precursors in the normal bone marrow.

Eosinophils can be enumerated in the peripheral blood either by 'wet counts' in modified Neubauer chambers, differential counts on dried smears or by automated cell counting. The automated counting that uses detection of eosinophil peroxidase is the most accurate method followed by counting in a cell chamber. Counting on smears is least accurate because of the tendency for eosinophils to congregate at the margins of the smear. It is preferable to record the eosinophil count in absolute numbers rather than as a percentage, as the latter will depend on the total cell count. The normal eosinophil count is (generally taken as) less than $0.4 \times 10^9/l$, although a study of 765 medical students in the USA measured counts ranging from 0.015 – $0.65 \times 10^9/l$.¹⁰⁹ It is higher in neonates. The eosinophil count varies with age, time of day, exercise and environmental stimuli, particularly allergen exposure. Blood eosinophil counts undergo diurnal variation, being lowest in the morning and highest at night. This effect resulted in a greater than 40% variation in one study.¹¹¹ This may be related to the reciprocal diurnal variation in cortisol levels which are highest in the morning.

The causes of an eosinophilia can be usefully classified according to the degree and frequency of occurrence (Table II). Division of eosinophil counts into degree is arbitrary but a mild eosinophilia could be regarded as 0.4 – $1.5 \times 10^9/l$, a moderate count as 1.5 – $5 \times 10^9/l$ and a high count as greater than $5 \times 10^9/l$. The commonest cause of an eosinophilia worldwide is infection with helminthic parasites, which can often result in a very high count. The commonest causes of an eosinophilia in industrialized countries are the atopic allergic diseases, seasonal and perennial rhinitis, atopic dermatitis and asthma. Allergic disease generally results in only a mild increase in eosinophil counts. A moderate or high eosinophil count in asthma raises the possibility of a complication such as Churg–Strauss syndrome or allergic bronchopulmonary aspergillosis (ABPA). Apart from allergic disease and helminthic parasites, a raised eosinophil count, especially a moderate or high count, is unusual.

Table II Causes of an eosinophilia

Disease	Frequency of cause of eosinophilia	Usual degree of eosinophilia	Comment
Infections			
Parasitic disease			
Bacterial	Common worldwide	Moderate to high	Usually cause eosinopenia, although serum ECP levels may be raised suggesting eosinophil involvement in tissue
Mycobacterial	Rare		More often secondary to drug therapy
Fungal	Rare		Apart from allergic reactions and coccidiomycosis in which as many as 88% of patients have an eosinophilia
Rickettsial infections	Rare		
Yeast	Rare		Cryptococcus reported as causing CSF eosinophilia
Viral infections	Rare		Occasional case reports of an eosinophilia in a variety of viral infections including herpes and HIV infection
Allergic diseases			
Allergic rhinitis	Common worldwide	Mild	Eosinophils seen in skin even with normal count
Atopic dermatitis	Common especially children	Mild	Syndrome of intrinsic asthma, nasal polyps and aspirin intolerance
Urticaria/angioedema	Common	Variable	associated with higher than usual eosinophil counts
Asthma	Common	Mild	
Drug reactions			
Many drugs	Uncommon	Mild to high	Count usually returns to normal on stopping
Neoplasms			
Eosinophil leukaemia	Rare	High	100 cases reported by 1988. Important to distinguish from hypereosinophilic syndrome (HES)
Myeloid leukaemia	Uncommon	Moderate to high	Raised eosinophil counts often seen in chronic myeloid leukaemia
Lymphomas	Uncommon	Moderate	Often intense tissue eosinophilia with moderate blood eosinophil count.
Histiocytosis X	Rare	Mild	Hodgkin's disease commonest type
Solid tumours	Uncommon	Mild to high	Intense tissue eosinophilia in eosinophilic granuloma but blood eosinophilia unusual Many different tumours reported

Table II Causes of an eosinophilia – continued

<i>Disease</i>	<i>Frequency of cause of eosinophilia</i>	<i>Usual degree of eosinophilia</i>	<i>Comment</i>
Musculoskeletal Rheumatoid arthritis Fasciitis	Rare Rare	Mild to high High	Occasional case reports. More usually secondary to therapy
Gastrointestinal Eosinophilic gastroenteritis	Rare	Mild to moderate	As with many gastrointestinal diseases there is often a marked tissue eosinophilia with only a mild or absent blood eosinophilia
Celiac disease	Uncommon	Normal	Tissue eosinophilia
Inflammatory bowel disease			Eosinophils seen in biopsies in both Crohn's and ulcerative colitis but blood eosinophilia unusual
Allergic gastroenteritis	Rare	Mild to high	Young children
Respiratory tract (for asthma, see Allergic diseases)			
Churg–Strauss syndrome	Rare	Moderate to high	Syndrome of eosinophilic vasculitis and asthma
Pulmonary eosinophilia	Uncommon	Mild to high	Syndrome of eosinophilia and chest X-ray shadowing, apart from ABPA*, usually of unknown cause
Bronchiectasis/cystic fibrosis	Uncommon	Mild	Often associated with asthma or ABPA
Skin diseases (for atopic dermatitis, see Allergic diseases)			
Bullous pemphigoid	Uncommon	Moderate	
Miscellaneous causes			
IL-2 therapy	Rare	Moderate to high	For renal cell carcinoma
HES	Rare	High	
Endomyocardial fibrosis	Rare	High	Secondary to any cause of a high eosinophil count
Hyper IgE syndrome	Rare	Moderate to high	
Eosinophilia/myalgia and toxic oil syndrome	Rare	High	Two related conditions one caused by poisoning with contaminated cooking oil in Spain and the other by a batch of tryptophan

+ allergic bronchopulmonary aspergillosis.

Eosinophils and disease

The role of eosinophils

Views on the role of eosinophils in health and disease have changed with time. For years they were thought to ameliorate inflammatory responses, now they are believed to have a tissue-damaging role.^{112,113} Even more recently it has become apparent that eosinophils are the source of a range of cytokines several of which are thought to have a homeostatic, rather than pro-inflammatory function. For example, the observation that eosinophils secrete TGF α together with studies showing increased numbers of eosinophils at the edges of healing wounds suggests that they may be important in wound healing.¹¹⁴ Cytokine-stimulated eosinophils secrete IL-1, express HLA class II receptors and present antigen to T cells *in vitro*, suggesting they may be important as accessory cells in T-cell-mediated reactions. There is evidence that eosinophils slow the rate of progression of solid tumours, presumably by being cytotoxic against tumour cells.¹¹⁵ Nonetheless, there is also little doubt that eosinophils can cause severe tissue damage under certain circumstances. Persistently high eosinophil counts from many causes including drug reactions, parasitic infections, eosinophilic leukaemia and hypereosinophilic syndrome are associated with endomyocardial fibrosis, a condition that presents with heart failure and signs consistent with a restrictive cardiomyopathy. The ventricle is thickened and histologically there are areas of fibrosis, thrombus formation and inflammation in the endomyocardium with large numbers of both intact and degranulating eosinophils. Eosinophil granule products are deposited adjacent to myocytes and *in vitro* have been shown to be toxic for cardiac myocytes. HES is a condition in which there is a high eosinophil count of unknown aetiology and it is associated with a number of features, several of which could be ascribed to the toxic properties of eosinophils.¹¹⁶

Much of the work undertaken in recent years on eosinophils has been in association with allergic disease and parasitic infection. The observation in the mid-1970s that eosinophils could kill parasite targets led to the hypothesis that the teleological role of eosinophils was to counter parasitic infection.¹¹⁷ The realization that eosinophils could release pro-inflammatory mediators such as PAF and eicosanoids, and the observation that eosinophil basic proteins were toxic for airway epithelium has led to a consensus that eosinophils are a major effector cell for tissue damage in asthma and could cause many of the pathological features of the disease. These conditions provide a useful model for eosinophil involvement in disease processes.

Eosinophils and asthma

It is well established that large numbers of eosinophils together with mononuclear cells are frequently found in and around the bronchi with patients who have died of asthma. The immunostaining of bronchial tissue from such patients has revealed the existence of large amounts of MBP deposited in the airways.¹¹⁸ The presence of increased numbers of peripheral blood eosinophils in both atopic and non-atopic chronic asthma is well known, although this elevation is not as great as that seen in other eosinophil-associated diseases and the peripheral blood eosinophil count is often normal.

Full appreciation of the extent of eosinophil involvement in asthma has come with the use of fiberoptic bronchoscopy to obtain BAL fluid and endobronchial biopsies from the airways in patients with mild to moderate asthma. Aerosolized challenge of sensitized asthmatics with allergen results in an influx of inflammatory cells consisting of eosinophils, neutrophils and mononuclear cells into the airways and an increase in the amount of eosinophil granule proteins in lavage fluid.¹¹⁹ A similar picture has been observed after challenge with agents that cause occupational asthma.^{120,121} The eosinophilia associated with segmental challenge down the bronchoscope is even more dramatic. Twenty-four hours after segmental challenge up to 50% of the lavage cells were eosinophils.¹²² Similar findings have been found after allergen challenge to the skin and nose.^{123,124}

An almost invariable increase in the number of eosinophils, in association with increased numbers of mast cells and epithelial cells has been observed in BAL fluid and endobronchial biopsies from clinical asthmatics compared with normal controls.¹²⁵⁻¹²⁸ A lesser, but often significant, increase in airway eosinophils is seen in atopic non-asthmatics or seasonal asthmatics out of season. Airway eosinophils in asthma are activated as determined by staining with mAb EG2 and expression of the activation receptor CD69.^{129,130} Eosinophil infiltration is accompanied by increased numbers of activated CD25-positive T-lymphocytes, which have a Th2-like profile of cytokine secretion¹³¹ and evidence of epithelial desquamation with increased numbers of epithelial cells in BAL fluid and signs of epithelial fragility in bronchial biopsies.¹³² The increase in eosinophils has been noted in intrinsic and occupational asthma as well as atopic asthma.^{133,134} A BAL eosinophilia is relatively specific to asthma, although it is also seen in pulmonary eosinophilia and some patients with fibrosing alveolitis.¹³⁵ The numbers of eosinophils in BAL fluid in asthma are generally only modestly raised ranging from 1% to 5% (normals, < 1%),

although occasionally eosinophil counts can be in the range of 30–50%. There is a general correlation between the numbers of airway eosinophils and the severity of asthma.¹³⁶ Inhibition of an airway eosinophilia by disodium chromoglycate (DSOG),¹³⁷ or more effectively corticosteroids,^{138,139} is associated with an improvement in bronchial hyperresponsiveness, symptoms and lung function. Inhibition of migration of eosinophils into the airways of allergen-challenged non-human primates, using an monoclonal antibody directed against the adhesion molecule ICAM-1, also inhibited the development of airway hyperresponsiveness.¹⁴⁰ However, none of these treatments is specific to the eosinophil. Glucocorticoids, for example, probably act to a large extent through inhibition of the release of eosinophil active cytokines from T-cells and monocytes.¹⁴¹ Airway eosinophilia can also occur without asthma or airways hyperresponsiveness.¹⁴² For eosinophils to cause tissue damage in the airways, they need to be actively secreting their mediators. Measurements of eosinophilic basic proteins may therefore be a better guide to the degree of eosinophilic inflammation than eosinophil numbers. For example, Adelroth and coworkers found that, whereas inhaled corticosteroids had no effect on the number of eosinophils in BAL fluid from asthmatics, they markedly reduced the amounts of ECP in lavage fluid.¹⁴³

Eosinophils and parasitic disease

Although infection with helminths is by far the commonest cause of a moderate to high eosinophilia in association with parasites, eosinophilia in association with protozoan infections has been described and ectoparasites such as head lice and scabies can produce a local eosinophilic reaction. The commoner helminthic causes of an eosinophilia are summarized in Table III.^{144–146} Eosinophils have been shown to be able to kill a number of opsonized parasites including newborn larvae of *T. spiralis*, larvae of *Nippostrongylus brasiliensis*, a gut parasite in the rat, and *F. hepatica* as well as schistosomulae of *S. mansoni*.¹⁴⁷ *In vivo* parasite larvae become coated with specific IgG and IgE antibodies and can activate complement. Dead larvae of *S. haematobium* and other parasites have been detected surrounded by eosinophils and eosinophil granule products in the skin.¹⁴⁸ Adult worms both *in vitro* and *in vivo* appear resistant to eosinophil-mediated damage. Despite this circumstantial evidence for eosinophils being involved in host defence against parasites, there remains some doubt about their role. Except for one study in the Gambia¹⁴⁹ there is no obvious correlation between the degree of eosinophilia and protection against infection or

reinfection. Moreover, treatment of mice infected with *N. brasiliensis* or *S. mansoni* with neutralizing anti-IL-5 mAbs abolished the eosinophilia without modulating the disease process.¹⁵⁰

Other eosinophilic disorders

More unusual eosinophilic disorders include pulmonary eosinophilia, idiopathic hypereosinophilic syndrome, eosinophil leukaemia and Churg–Strauss syndrome, which is a life-threatening condition characterized by eosinophilic vasculitis, asthma and a peripheral blood eosinophilia.¹⁵¹ These conditions are of unknown aetiology. Management generally consists of treatment with high-dose oral glucocorticoids supplemented by chemotherapy, if the condition is only partially responsive, as is generally the case in HES and Churg–Strauss syndrome. More recently therapy with interferon α and γ has been used with anecdotal success in HES. IL-5 antagonists, when they become available, may also be effective. Two interesting and related conditions that have been described recently are toxic oil syndrome (TOS) and eosinophilia–myalgia syndrome.¹⁵² Eosinophilia–myalgia syndrome was first described in October 1989 in New Mexico and 1,500 cases were reported by mid-1990 with 27 deaths. It was caused by ingestion of a batch of tryptophan and thought to be due to a contaminant possibly 1,1'-ethylenedibis (tryptophan). It was characterized by severe myalgia in association with an eosinophil count of greater than $1 \times 10^9/l$ with a median count of $4-6 \times 10^9/l$. Patients also complained of fatigue, shortness of breath, cough, rash and headache. Histopathologically there was a perivascular lymphocyte and eosinophilic infiltrate in the dermis, fascia and skeletal muscle with a pulmonary vasculitis and alveolitis. The histological appearances were similar to eosinophilic fasciitis. Symptoms were persistent and not very responsive to treatment with glucocorticoids and immunosuppressants. TOS was an epidemic in Spain in 1981 caused by the ingestion of industrial rapeseed oil. A total of 20,000 people were affected with 300 deaths. The acute phase of TOS was characterized by an adult respiratory distress syndrome (ARDS) like picture with a profound eosinophilia but the chronic phase resembled eosinophilia–myalgia syndrome both clinically and histopathologically. Fifty per cent of patients had complete resolution after 8 years. The precise aetiological agent is unknown.

Eosinopenia

The normal eosinophil count is often low, although in one study of over 20,000 patients only 24 had

Table III Helminthic causes of eosinophilia

Parasite	Comment
Nematodes	
Ascariasis	Higher eosinophil counts in children. Larvae migrate from intestine to lungs where they cause Loeffler's syndrome, a form of pulmonary eosinophilia.
<i>Toxocara canis</i>	Infective eggs are present in faeces of puppies and pregnant bitches. Larvae in hosts such as chicken. Eosinophilia seen mainly in children under 9 years. Can migrate to eye and cause blindness. Serological evidence suggests infection not uncommon in industrialized countries.
Filariasis	Common. Invariably result in marked eosinophilia especially Loa Loa infection. Filiriasis is the cause of tropical pulmonary eosinophilia due to migration of adult worms to lung, elephantiasis due to involvement of lymphatics (<i>Wuchereria bancrofti</i> and <i>Brugia malayi</i>) and river blindness (<i>Onchocerca volvulus</i>). Treatment can result in systemic reaction called Mazzotti reaction possibly due to massive eosinophil degranulation.
Ancylostomiasis	Hookworm infection. <i>Ancylostoma duodenale</i> and <i>Necator americanus</i> . One of the main causes of eosinophilia in patients returning from tropical countries. Counts in region of $2 \times 10^9/l$.
Strongyloidiasis	Subclinical infection can persist over 20 years. Stool examinations often negative. Cause of eosinophilia in ex-servicemen who spent time in tropics. If <i>Strongyloides</i> infection is not considered and these patients are given steroids for suspected HES, or as trial of therapy, they can develop disseminated disease.
Trichinosis	Caused by ingestion of encysted muscle larvae of <i>Trichinella spiralis</i> . Most prominent eosinophilia seen during early stages of infection when larvae migrating into striated muscle via the blood. Fatal cases reported, of which only 20% were noted to have an eosinophilia.
Others	Other nematodes that can cause eosinophilia include <i>Trichuris trichiura</i> , <i>Capillaria</i> and <i>Gnathostomiasis</i> . The thread worm <i>Enterobius vermicularis</i> occasionally causes eosinophilia when it invades tissues.
Trematodes	
Schistosomiasis (Bilharzia)	Infection with one of the <i>Schistosoma</i> (blood flukes), <i>S. mansoni</i> , <i>S. haematobium</i> and <i>S. japonicum</i> , is perhaps the commonest cause of a moderate to high eosinophilia worldwide with 200 million people being infected. Infection is nearly always associated with an eosinophilia.
Fascioliasis	Adult worms of <i>F. hepatica</i> reside in the bile ducts where they are associated with abnormal liver function tests and an eosinophilia.
Cestodes	
<i>Echinococcus</i>	Eosinophilia occurs in 25–50% of patients with hydatid disease.

counts of less than $0.01 \times 10^9/l$. In each case this could be ascribed to the disease or treatment.³ Acute infections, treatment with glucocorticoids and adrenaline decrease eosinophil counts. In contrast, β blockers inhibit adrenaline-induced eosinopenia and can cause a rise in the eosinophil count. The mechanism by which eosinopenia occurs in these circumstances is not fully understood. Beeson and Bass found that acute infection in mice resulted in a rapid fall in eosinophil counts due to either margination or migration into tissue and a more prolonged eosinophilia due to inhibition of bone marrow production. A soluble mediator of greater than 30,000 kDa appeared to be involved.¹⁵³ There have been several isolated case reports of patients with absent eosinophils in the blood and bone marrow.¹⁵⁴ Often the cause of the eosinopenia was related to some immunological problem. For example, in one case it occurred after drug-induced agranulocytosis¹⁵⁵ and in another there was a serum inhibitor of eosinophil colony formation.¹⁵⁶ A rare disorder is EPO deficiency, cases of which may be brought to light by automatic counting that uses detection of EPO to count eosinophils. EPO deficiency does not appear to have any adverse clinical consequences.¹⁵⁷

Summary and conclusions

Eosinophils are characterized by their unique crystalloid granules that contain four basic proteins – MBP, ECP, EDN and EPO. The cell has many common features with neutrophils but, unlike that

cell type, eosinophils utilize VLA-4/VCAM-1 as an adherence pathway and have a number of other receptors not shared by neutrophils. These include recognition units for IgE (distinct from CD23), and receptors for IL-5, IL-3 and RANTES. Following stimulation with a variety of agents, eosinophils preferentially elaborate LTC₄ as the major 5-lipoxygenase product of arachidonic acid and produce substantial amounts of PAF. Of particular interest is the ability of eosinophils to synthesize a number of cytokines. Thus eosinophils have marked pro-inflammatory potential.

There is now convincing evidence that eosinophilia is T-cell dependent. The Th2-type cell, which selectively secretes IL-5 and IL-4, seems particularly involved. IL-5, IL-3 and GM-CSF are required for eosinophil maturation, and cause activation and prolonged survival of the mature cell. IL-5 is unique in that it promotes terminal differentiation of the committed eosinophil precursor and *in vivo* in mice appears to be sufficient on its own for eosinophil growth from uncommitted stem cells. IL-4 selectively upregulates VCAM-1 expression on endothelial cells thus augmenting VLA-4-dependent eosinophil adhesion. The role of eosinophils in disease is complex but in general their numbers are increased in helminthic parasitic disease and atopic allergy and asthma. Eosinophil products can produce many of the pathological features of asthma, and helminthic larvae coated with immunoglobulin or complement are particularly susceptible to eosinophil-mediated cytotoxicity. Eosinopenia is often related to acute inflammation or stress.

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