Eosinophil trafficking in asthma

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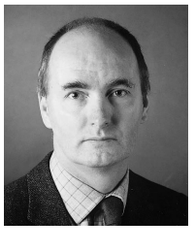

This article is based on the regional lecture given at Charing Cross Hospital on 12 July 2000 by **Andrew J Wardlaw** MD PhD, Professor in Respiratory Medicine, Division of Respiratory Medicine, Institute for Lung Health, Leicester-Warwick Medical School, Leicester

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ABSTRACT – Asthma is characterised by a 50–fold increase in the number of eosinophils relative to neutrophils in the bronchial mucosa. This is the result of the cumulative and sequential effects of several, approximately fourfold, increases in selective eosinophil versus neutrophil migration occurring at a number of stages in the life cycle of the eosinophil. These events, which are integrated and directed by allergen-specific T helper 2 lymphocytes through the generation of interleukin (IL)-5, IL-4 and IL-13, include:

- **effects on the bone marrow, mediated principally by IL-5, which result in a fourfold increase in circulating eosinophils**
- **selective tethering of eosinophils to venular endothelium through the combined effects of P-selectin/P-selectin glycoprotein ligand (PSGL)-1 and very late activation antigen (VLA)-4/vascular cell adhesion molecule-1, which has the potential for an up to tenfold increase in eosinophil versus neutrophil adhesion**
- **selective chemotaxis under the influence of CC chemokines**
- **prolonged survival, again mediated by IL-5.**

The implications of this multistep process are that antagonists of IL-5, VLA-4, PSGL-1 and CC chemokine receptor 3, as well as IL-4 and IL-13, each have the potential markedly to inhibit eosinophil recruitment in asthma.

Allergic diseases such as asthma are characterised by increased numbers of eosinophils in the affected tissue[1]. This increase in eosinophils has a degree of selectivity in that it usually occurs without an increase in neutrophils. This observation is one of the cornerstones of the current hypothesis suggesting a central role for eosinophil derived mediators in causing asthma and related allergic diseases. This review attempts to summarise, in terms of a coherent framework, the fruits of the major research effort that in recent years has tried to explain the molecular basis for selective eosinophil migration[2] (Fig 1). Apart from lymphocyte homing, selective eosinophil accumulation is perhaps the best studied model of how different patterns of cell accumulation occur in various inflammatory diseases. As a result, it offers insights into how selective leucocyte trafficking may occur in other pathological processes.

Early thoughts on eosinophil trafficking into tissues were dominated by the idea of a selective chemoattractant. An activity, termed eosinophil chemotactic factor of anaphylaxis (ECF-A), was detected in supernatants from anaphylactically challenged guinea pig lung and appeared to be selectively chemotactic for eosinophils. This was subsequently found to consist of a mixture of leukotriene B4, which is active on guinea pig eosinophils but less so on human eosinophils, and 18(s),15(s)-dihydroxy-eicosatetraenoic acid[3]. ECF-A from human lung was later identified and characterised as two tetrapeptides, Val-Gly-Ser Glu and Ala-Gly-Ser Glu. However, the later characterisation of effective ECFs such as platelet activating factor (PAF) revealed that the ECF-A tetrapeptides had negligible activity[4]. Indeed, there is limited evidence that anti-immunoglobulin E-challenged human lung mast cells are an important source of selective eosinophil chemoattractants. The effects of these cells on eosinophil migration are probably largely indirect through generation of cytokines such as tumour necrosis factor (TNF)α, interleukin (IL)-4 and IL-13. In the late 1980s attention turned towards the importance of IL-5 as well as the possible role of adhesion pathways in controlling selective eosinophil accumulation. More recently, the discovery of chemokines has revived interest in the central role of selective chemoattractants in directing eosinophil migration.

How selective is eosinophil migration in asthma?

Selective accumulation of eosinophils in the airways in asthma has become a central tenet of the pathology of the disease. This is based on a number of different types of studies, including:

- post-mortem analysis of the pathology of asthma deaths
- studies of induced sputum
- the use of fibre-optic bronchoscopy to obtain endobronchial biopsies
- bronchoalveolar lavage[5].

Most of these studies have demonstrated a significant increase in the number of airway eosinophils

compared with appropriate controls, without a corresponding increase in airway neutrophilia. The airway eosinophilia is variable but generally fairly modest, and differs from non-asthmatic subjects because of the paucity of eosinophils in normal airways. Eosinophils usually make up about 3% of the leucocytes in asthmatic bronchoalveolar lavage (similar to neutrophils and lymphocytes). Data from a range of studies of clinical asthma show that eosinophils are generally 2–4 times more numerous than neutrophils in bronchial biopsies, whereas in the normal airway neutrophils are 10–50 times more numerous than eosinophils, giving a 20–600 fold increase in the number of eosinophils relative to neutrophils in normal versus asthmatic airways.

Eosinophil trafficking

Eosinophils differentiate from bone marrow precursors under the influence of growth factors, including IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF), which are active on early precursors, and IL-5 which acts as a late differentiation factor[6]. In humans, IL-5 appears to be active only on eosinophils and basophils. IL-5 production is increased in asthma and may act hormonally on the bone marrow to increase eosinophilopoiesis. There is also evidence for increased numbers of circulating eosinophil precursors in the peripheral blood of allergic patients which may be able to migrate into the lung and differentiate *in situ*[7].

IL-5 is needed to mount an eosinophilic response in allergic disease. Antibodies against IL-5 in a number of animal models have prevented the peripheral blood and airway eosinophilia associated with antigen challenge. IL-5 gene-deleted mice were unable to mount an eosinophilic response to allergic stimuli, although eosinophil production was not completely ablated[8]. IL-5 can also prolong the survival of mature eosinophils. In addition, although not chemotactic in its own right, IL-5 is a potent enhancer *in vitro* of the chemotactic effects of established eosinophil chemoattractants. Anti-IL-5 antibodies markedly diminish airway eosinophilia after allergen challenge in humans (although without obvious effect on lung function). The greater numbers of eosinophils in the blood of allergic individuals is a combination of increased haematopoiesis and rate of egress (Fig 1). Eotaxin, a specific eosinophil chemoattractant, and IL-5, given intravenously cause a synergistic increase in emigration of eosinophils from the bone marrow[9], emphasising the co-operative effects of IL-5 and a chemotactic stimulus on eosinophil locomotor behaviour.

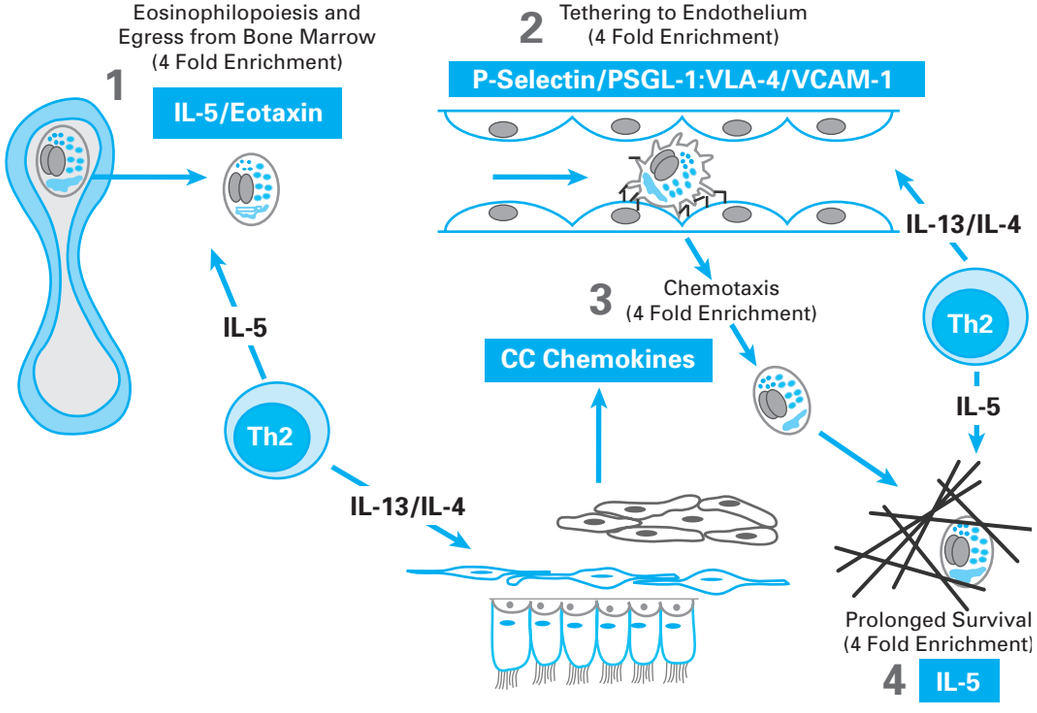

Fig 1. Schematic representation of the multi-step paradigm of eosinophil recruitment into tissue. Selective accumulation of eosinophils occurs as approximately fourfold sequential and cumulative increases in eosinophils compared to neutrophils at several stages in the life cycle of the cell. Each step is under separate molecular control, influenced either directly or indirectly by T helper (Th) 2 cytokine production: **step 1** involves haematopoiesis and bone marrow egress mediated by interleukin (IL)-5 and chemotactic signals; **step 2** is through IL-4 and IL-13 upregulation of P-selectin and vascular cell adhesion molecule (VCAM)-1 on vascular endothelium; **step 3** involves selective chemotaxis under the influence of CC chemokines generated by IL-4 and IL-13-stimulated epithelial, fibroblast and smooth muscle cells; **step 4** is prolonged survival, again mediated by IL-5 (PSGL = P-selectin glycoprotein ligand; VLA = very late activation antigen).

Integrin receptors are also involved in regulating eosinophil migration into the blood. Integrins are a large family of two-chain (α,β) membrane receptors that are widely expressed on a range of leucocytes and tissue cells. Most integrins bind to matrix proteins, but a few bind to ligands on endothelium and control leucocyte transmigration. The 'leucocyte integrins', in particular Mac-1 and lymphocyte function associated antigen (LFA)-1 which bind intercellular adhesion molecule (ICAM)-1, are crucial to migration of all leucocytes, whereas the very late activation antigen (VLA)-4 integrin, which binds vascular cell adhesion molecule (VCAM)-1, is not expressed by neutrophils. In the bone marrow, egress was accelerated by an antibody against VLA-4, whereas an antibody against Mac-1 prevented egress, showing that adhesion to sinus endothelium is also important in controlling emigration.

Adhesion

Whatever the percentage of eosinophils in the blood, accumulation of eosinophils in the airway will not occur unless there are local signals on bronchial post-capillary endothelium leading to adhesion and transmigration. Successful transmigration requires a number of events to occur in series. The leucocyte flowing into the post-capillary venule must be 'captured' by the endothelium, a process mediated by two families of adhesion receptors: the selectins (calcium-dependent (C type) lectins that bind carbohydrate) and their ligands, and the VLA-4 binding to VCAM-1 (Fig 2).

Once tethered, the cells then roll along the surface of the endothelium until they become activated by chemoattractants. Activation allows engagement of the leucocyte integrins Mac-1 and LFA-1 which results in firm arrest, a prerequisite for transmigration[10]. This fundamental paradigm of the way in which leucocytes migrate into tissue permits a great deal of diversity in the pattern of signals controlling accumulation of specific cell types.

VLA-4 is not expressed on human neutrophils, so it has attracted considerable interest as a possible receptor mediating selective eosinophil adhesion. IL-4 and IL-13, two cytokines expressed by T helper (Th) 2 lymphocytes, and therefore closely linked to asthma, enhance migration of eosinophils by increasing VCAM-1 expression on endothelial cells. There is also increasing evidence for a role for P-selectin, one of the selectins expressed on endothelium and in platelets, in mediating eosinophil adhesion. Eosinophils bind better than neutrophils to purified P-selectin because they express more of the ligand P-selectin glycoprotein ligand (PSGL)-1. This is particularly obvious at the low concentrations of P-selectin expressed on the blood vessels in asthma[11,12]. IL-4 and IL-13 increase expression of P-selectin as well as VCAM-1[13,14]. P-selectin was the major receptor involved in eosinophil adhesion to nasal polyp endothelium as a model of asthma[15]. The reduced eosinophil infiltration in nasal polyps after treatment with fluticasone was associated with a reduction in P-selectin expression[16]. Eosinophil accumulation was also less in the airways of P-selectin deficient mice after antigen challenge[17].

In summary, VLA-4/VCAM-1 and P-selectin/PSGL-1 co-operate at the tethering step to bind eosinophils, but not neutrophils, to airway endothelium when exposed to IL-4 and IL-13 (cytokines produced in large amounts in asthma).

After leucocytes have been captured by the endothelium, they need to become activated. This allows a change in the structure of Mac-1 and LFA-1 which then can bind their ligand ICAM-1. This stops the cell from rolling, bringing it to a halt. Only then can it move through the blood vessel wall. The activation step is mediated by chemoattractants expressed on the endothelial surface[18]. IL-8 and PAF are thought to be important in activating neutrophils, whereas the chemoattractant eotaxin (and other related molecules) are thought to cause eosinophil arrest. Eotaxin binds to the CC chemokine receptor CCR3, which is expressed only by eosinophils and basophils and is thus a good target to block eosinophil migration.

Selective chemoattractants

Until this last decade, most effective eosinophil chemoattractants which had been identified, such as PAF and C5a, were also active on neutrophils. In 1992, RANTES, a member of a new family of peptide chemoattractants termed chemokines, was shown to be an effective eosinophil chemoattractant which was not active on neutrophils. A number of other selective and highly effective eosinophil chemokines have since been discovered, most of which bind through CCR3. They include eotaxin 1, 2 and 3 and monocyte chemoattractant protein 2, 3 and 4. Increased expression of a number of these chemokines, including eotaxin and RANTES, has been demonstrated in asthma[19]. In some cases expression has correlated with

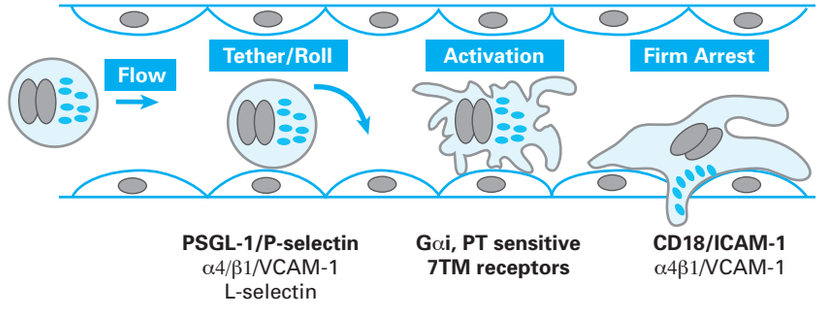

Fig 2. Schematic representation of the steps mediating eosinophil adhesion to vascular endothelium. Eosinophils enter the post-capillary endothelium under flow conditions, and become tethered to the endothelium through the combined effects of very late activation antigen (VLA)-4/vascular cell adhesion molecule (VCAM)-1, P-selectin glycoprotein ligand (PSGL)-1/P-selectin and, in some circumstances, L-selectin. Activation, possibly through chemoattractant receptors, results in binding to intercellular adhesion molecule (ICAM)-1 of CD18 integrins lymphocyte function associated antigen (LFA)-1 and Mac-1, which are the major receptors involved in transmigration, although VLA-4/VCAM-1 also makes a contribution at this stage.

eosinophil counts. Low levels of constitutive expression have usually been detected in normal control subjects.

Several animal models have been used to study the role of chemokines (as well as growth factors and adhesion receptors) in asthma, in particular ova sensitised and challenged mice. The mouse model is particularly powerful because of the ability to study modifications of the gene of interest – although substantial differences in the anatomy of the mouse and human lung caution against overinterpretation of the data, particularly in terms of the relationship between inflammatory changes and bronchial hyperresponsiveness. Evidence for a role for most of the CCR-3 binding chemokines, particularly eotaxin, has been obtained using animal models[20]. However, as might be expected, complete abrogation of eosinophil migration has not been observed by eliminating any single chemokine. Results with a mouse in which the CCR3 gene has been disabled, where a more profound effect might be expected, are awaited with interest. CC chemokines seem to be produced mainly by structural cells such as the bronchial epithelium or smooth muscle. In this context, it is particularly interesting that IL-4 and IL-13 stimulated the production of eotaxin from fibroblasts and epithelial cells, so linking eosinophil chemoattractant release with Th2-related immunological events[21].

Prolonged survival

Eosinophils rapidly undergo apoptosis unless provided with support from eosinophil growth factors such as IL-3, IL-5 and GM-CSF, each of which is present in increased amounts in the airways of asthmatics. The signalling pathways involved in growth factor-induced eosinophil survival are complex and involve a number of signal transduction molecules which can phosphorylate other proteins on the amino acid tyrosine (therefore called tyrosine kinases). These include the Lyn, Jak 2, Raf 1 and MAP kinases[22]. IL-5 delays apoptosis by inhibiting the translocation of the protein BAX to the mitochondria. BAX in the mitochondria causes it to become leaky, and this initiates the apoptosis cascade[23]. Direct evidence for prolonged survival was provided by a study in which anti-IL-5 antibodies caused rapid loss of eosinophils from cultured explants of nasal polyps[24]. The number of apoptotic eosinophils in the airways of asthmatics was increased in subjects treated with inhaled glucocorticoids[25].

The multistep paradigm of selective eosinophil recruitment: implications for drug development

Estimation from various bronchoscopy studies in asthma suggest that there is an approximately 50–fold increase in the accumulation of eosinophils over neutrophils in the airways in clinical disease. Increased neutrophil migration may also occur in some individuals, so the total increase in eosinophil trafficking is likely to be even greater – but it is the mechanisms of selective trafficking with which this review is particularly concerned. The relative contribution of each stage in the life cycle of the eosinophil to selective trafficking probably varies both between and within individuals at different times in the disease process. The effect of increased haematopoiesis and release from the bone marrow on selective eosinophil migration can be calculated from the peripheral blood eosinophil count. It varies greatly but, in terms of both percentage and total numbers, is about four times higher in asthma than in normal subjects.

Adhesion to endothelium is an absolute requirement for migration. The contribution of adhesion to selective eosinophil recruitment will depend crucially on the relative amount of specific allergy-related cytokine such as IL-4 compared with general stimulatory cytokines such as TNFα that are generated. In our experiments, a striking finding was that up to ten times more eosinophils than neutrophils are bound to nasal polyp endothelium, supporting the idea that the combined effect of bone marrow and endothelial adhesion events could therefore easily result in about twenty times as many eosinophils as neutrophils tethered to the bronchial endothelium in asthma.

Eosinophil chemoattractants, particularly chemokines, are clearly important in directing eosinophils into tissue. However, the expression of effective neutrophil chemoattractants, particularly IL-8, in allergic disease is increased, so any neutrophils which adhere to the bronchovascular endothelium in asthma should be able to migrate efficiently into the airway submucosa. The effect of the chemotaxis step on selective trafficking of eosinophils versus neutrophils may therefore be not as great as has been suggested by the widely used animal models in which relatively few neutrophils are recruited. How much selectivity occurs at the chemotaxis stage in human asthma is therefore guesswork, but estimates of fourfold may be taken for illustrative purposes and, similarly, a fourfold effect ascribed for enrichment of prolonged survival. The exact numbers do not matter other than to illustrate that each stage can have a marked effect on selective recruitment and that it is unlikely that any single stage, let alone any single molecule, is wholly responsible. The cumulative effects of each stage are more than enough to result in the enrichment of eosinophils seen in disease (Fig 1). Although multiple molecular events direct recruitment, these events are integrated and controlled by the cytokines IL-5, IL-4 and IL-13[26]. In atopic disease at least, these cytokines are likely to be largely generated in a co-ordinate fashion by allergen stimulated CD4+ve Th2 lymphocytes.

What does this multistep process mean for the development of drugs to inhibit eosinophil recruitment? An important feature of the concept outlined above is that it should be possible to inhibit recruitment at each of the stages. Antagonists of IL-5,

Key Points

Asthma is characterized by an increase in the number of eosinophils relative to neutrophils in the affected tissue.

An enormous research effort has occurred over the past 30 years in an attempt to understand the molecular basis for selective eosinophil recruitment in asthma.

Several drugs that will inhibit accumulation of eosinophils in tissue should become available in the next 5 years.

P-selectin, VLA-5 and CCR3 should all inhibit eosinophil accumulation relatively selectively. An alternative strategy would be to suppress the production of Th2 associated cytokines which orchestrate eosinophil migration[27].

In summary, the enormous effort that has gone into understanding the molecular basis for selective eosinophil recruitment in asthma and related diseases over the last 30 years has borne fruit. In the coming five years several drugs should be available which will more or less specifically inhibit accumulation of eosinophils in tissue. At the very least, this will help to answer one of the central questions that has concerned eosinophil biologists for the last three decades. Do eosinophils really cause asthma?

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