

EDITORIAL

Eosinophil trafficking: new answers to old questions

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Summary: Selective tissue accumulation of eosinophils is one of the hallmarks of allergic disease. The mechanisms controlling eosinophil trafficking involve a combination of IL-5-dependent eosinophilopoiesis, regulation of adhesion and chemoattractant-dependent interactions with vascular endothelium and control of the fate of the eosinophil in tissue by the integration of adhesion and chemotactic signals delivered by matrix proteins and structural cells. Two papers in this issue of the journal add further information. One study reports anti-thrombin III as a new chemoattractant binding to Syndecan-4 and a second study reveals the release of granulocyte macrophage-colony stimulating factor and regulated on activation, normal T cells expressed and secreted by rhinovirus-infected tracheal submucosal glands. The way in which these new observations fit into the eosinophil trafficking jigsaw will be discussed.

A characteristic and histologically striking feature of allergic diseases such as asthma and allergic rhinitis is the up to 100-fold increase in the accumulation of eosinophils at inflammatory sites. This finding is distinctive not least because in the normal state these cells are largely absent from tissues other than the gut where constitutive migration is controlled by eotaxin and $\alpha 4\beta 7$ binding to MAdCAM-1 [1]. A tissue eosinophilia is often a relatively selective event without any increase in other leucocyte subsets such as T cells and neutrophils. Indeed together with lymphocyte homing eosinophil accumulation is one of the best-studied models of the molecular mechanisms involved in selective leucocyte migration, reviewed in [2–4] (Fig. 1).

Selective pressure on eosinophil accumulation occurs at every stage in the life cycle of the eosinophil. IL-5 is crucially important as a selective growth factor and together with eosinophil-specific chemoattractants such as eotaxin can cause egress of eosinophils from the bone marrow compartment without affecting other leucocytes [5]. The post-capillary venular endothelium is a major control point for leucocyte migration involving a series of obligate steps each controlled by distinct families of adhesion receptors and chemoattractants, which allows for considerable combinatorial diversity [6]. The capture step involving selectins and $\alpha 4$ integrins appears to be of particular importance in controlling eosinophil migration. The modest but specific increase in endothelial P-selectin and VCAM-1 expression on endothelial cells induced by IL-4 and IL-13 are sufficient to mediate adhesion of eosinophils but not neutrophils under physiological flow conditions through binding to P-selectin glycoprotein 1 (PSGL-1) and VLA-4 ($\alpha 4\beta 7$) [7, 8]. This is because higher levels of PSGL-1 expression on eosinophils result in greater avidity for P-selectin than neutrophils, particularly at the low levels of P-selectin expression induced by the Th2 cytokines and because human neutrophils do not ex-

press VLA-4 [9–11]. Eosinophils bind relatively poorly to E-selectin, expression of which is induced by Th1-associated cytokines such as IFN- γ and TNF- α and unaffected by IL-4 or IL-13 [12]. The contribution of the activation step in selective eosinophil adhesion to endothelium remains uncertain. The paradigm of leucocyte trafficking anticipates that eosinophils should arrest as a result of exposure to endothelial chemoattractants. These include chemokines binding to eosinophil chemokine receptors, in particular CCR3. Although eosinophils will readily go from a rolling to an arrested phenotype on cytokine-activated endothelium under flow conditions when stimulated exogenously with chemoattractants the evidence for an endogenous chemoattractant is less clear cut. In particular pertussis toxin (PT), which inhibits most chemoattractant-mediated activation events, and an anti-CCR3 mAb only partially inhibited eosinophil arrest on endothelium under flow conditions [13]. In our hands eosinophils continue to roll on cytokine-activated endothelium under flow conditions unless exogenously stimulated (personal observation) although not all investigators have found this [8, 14]. In a recent study eotaxin 3, one of the several ligands for CCR3, was involved in eosinophil transmigration through IL-4-stimulated endothelium [15]. Interestingly however in the CCR3 gene-deleted mouse the eosinophils appeared to be able to migrate through the endothelial cells, but not the endothelial basement membrane suggesting that in the mouse model of asthma CCR3 binding chemokines are important in migration into tissue but not in adhesion and transmigration through endothelium [16]. Lymphocyte function antigen-1 and Mac-1 binding to intercellular adhesion molecule (ICAM)-1 and ICAM-2 play essential roles in the arrest of eosinophils on endothelium and in transmigration. However, this pathway is shared by all other leucocyte subsets [17]. VLA-4/VCAM-1 is also involved in eosinophil arrest on TNF- α -stimulated endothelium [14], but there appears to be no major bias in the binding of subsets of leucocytes to TNF- α -stimulated endothelium [18].

Transmigration of leucocytes requires active involvement of the endothelium with remodelling of tight junctions to allow egress of the cell. The leucocyte also has to penetrate the endothelial basement membrane, probably by activation of metalloproteinases such as MMP-9 [19]. There is no evidence for eosinophil selective components to these events, but they have not been intensively studied. In the lung, migration into the bronchial mucosa is through the systemic bronchial circulation. However, migration into the alveolar compartment that is relevant to interstitial lung diseases such as eosinophilic pneumonia is through the low pressure pulmonary vasculature where the capture step is less important, particularly in the capillary bed and is more likely to be dependent on chemoattractants alone. Consistent with this selectins are not

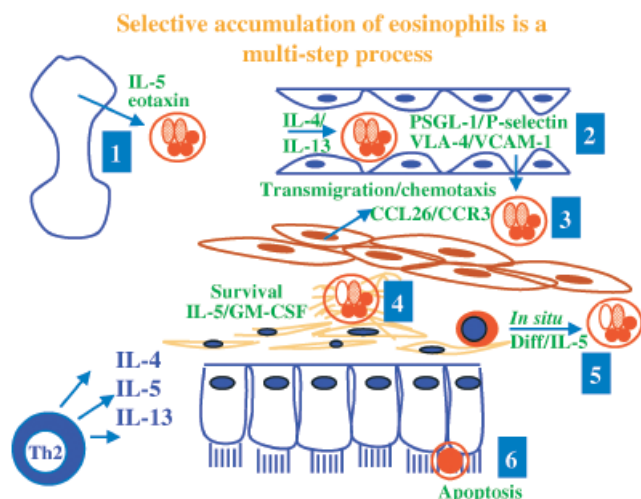


Fig. 1. Eosinophil accumulation involves several steps with multiple points at which selective migration can occur. The first step (1) involves eosinophilopoiesis in the bone marrow under the influence of IL-5 followed by egress through the bone marrow sinusoidal endothelium which is mediated in part by eosinophil chemoattractants. P-selectin /PSGL-1 and VLA-4/VCAM-1 are particularly important in controlling selective egress of eosinophil through post-capillary endothelium (2) with CCR3 binding chemokines particularly eotaxin 3 (CCL27) involved in transmigration (3). In the tissue growth factor mediated prolongation of survival, in part brought about by interaction with structural cells and matrix proteins such as fibronectin are important in tissue retention (4). *In situ* differentiation from pre-cursors may also increase tissue eosinophil numbers (5). Cells become apoptotic and are removed as they migrate out into the lumen (6). These processes are controlled by the Th2 associated cytokines IL-4, IL-13 and IL-5.

expressed on the pulmonary capillary circulation. Certainly animal models of neutrophil migration into the alveolar compartment stimulated by *Streptococcus pneumoniae* or lipopolysaccharide have been only partially inhibited by blocking selectins or leucocyte integrins and their ligands [20].

One feature of the mechanisms that controls selective eosinophil accumulation is the integration of the process by the Th2 associated cytokines IL-5, IL-4 and IL-13. These are central to the kinetics of eosinophil trafficking both directly as discussed above and indirectly by enhancement of the generation of CCR3 binding chemokines by structural cells [21]. Eosinophil accumulation in inflammatory disease is generally lymphocyte dependent [22] and it is these cytokines that make a direct link between antigen specific T cell activation and eosinophil accumulation. This would support the view that inhibition of Th2 cytokines would prevent eosinophil migration and the studies with anti-IL-5 discussed below offer conditional support for this. However it should be remembered that inflammatory processes that apparently involve Th1 associated events favouring the release of TNF- α , IL-1 and IFN- γ can also promote eosinophil migration, although in a non-specific manner. One might speculate that in some forms of allergic disease such as mild atopic asthma Th2 activation plays a prominent role whereas in more severe disease such as acute severe asthma, Th1 related events may also be involved.

Of equal importance as endothelial interactions to the kinetics of eosinophil migration are the factors controlling the fate of the eosinophil once it enters the tissue. There are three possible outcomes. The eosinophil can remain in the tissue interacting with matrix proteins, other leucocytes or structural cells such as, in the bronchial mucosa, the epithelium,

airway smooth muscle, mucus glands and nerves; alternatively the cell can migrate into the lumen of the gut or airway where it is likely to undergo apoptosis and be removed; or it can return to the circulation via the lymphatics. There is limited evidence that eosinophils can re-circulate although they have been reported to be present in lymph nodes where it has been speculated that they are involved in antigen presentation [23]. The length of time that eosinophils remain in tissue before migrating into the lumen is unclear as there are virtually no studies of the kinetics of eosinophil migration *in vivo* in humans. However, the studies using anti-IL-5 in which there remained a significant tissue eosinophilia even after the blood eosinophilia was almost completely inhibited would suggest that eosinophils can remain in the tissue for at least 12 weeks [24]. Anti-IL-5 also completely inhibited migration into the lumen, which suggests that trans-epithelial migration is IL-5 dependent. It is of interest that in the mouse model of asthma eosinophil migration into the lumen did not occur in the MMP-2 gene-deleted mouse, which caused the animals to asphyxiate [25]. IL-5 may therefore be important in activating the eosinophils to digest the epithelial basement membrane in a MMP-2-dependent manner [26]. Consistent with egress of eosinophils into the lumen being important in their removal eosinophils do not appear to undergo apoptosis in tissue but only when they migrate into the lumen [27]. It is interesting to speculate that, as has been shown with senescent neutrophils, when tissue eosinophils become senescent they start to alter their receptor phenotype in a way that inhibits tissue retention and promotes migration into the lumen [28]. The factors controlling the retention and survival of eosinophils in tissue are likely to involve the integration of chemoattractant, adhesive and survival signals delivered by interactions with matrix proteins and structural cells. Although IL-5 may play a role [29], the anti-IL-5 studies show this cytokine is at least partly redundant in this process. The autocrine release of granulocyte macrophage-colony stimulating factor (GM-CSF) by the eosinophil in response to outside in signals delivered through adhesion to fibronectin and other matrix proteins is likely to be important in eosinophil tissue retention [30].

Although a number of CC chemokines including RANTES, eotaxin 1–3, macrophage chemotactic protein-1–4 and mucosal epithelial chemoattractant have been shown to be effective and relatively selective eosinophil chemoattractants, many of which are generated in allergic disease [31], a plethora of other potent and effective eosinophil chemoattractants have been described although most of these are also active on neutrophils and in some cases monocytes. These include lipids such as platelet activating factor and 5-oxoETE, and C5a and C3a [32]. Other agents such as leukotriene E₄ are effective in mediating eosinophil accumulation *in vivo* even though they are not directly chemotactic [33]. For the most part the role these mediators play in eosinophil accumulation in disease remains uncertain. *In vitro* or even *in vivo* activity does not mean a mediator is relevant to disease as was shown by the disappointing effects of platelet activity factor antagonists and the limited effect of leukotriene antagonists on eosinophilic inflammation in asthma.

In this issue of *Clinical and Experimental Allergy* there are two further reports of eosinophil active mediators. What role

may they play in eosinophil accumulation? The paper by Feistritz et al. [34] reporting the chemotactic effect of anti-thrombin III (AT-III) appears to be of particular relevance to the events controlling eosinophil transmigration, whereas the paper by Furukawa et al. [35], demonstrating chemotactic factors released by airway submucosal glands is perhaps more related to the fate of eosinophils in tissue. Feistritz et al. have demonstrated that AT-III is chemotactic for eosinophils and that the receptor on eosinophils is the heparan sulphate proteoglycan (HSPG) Syndecan-4. Interaction of AT III with Syndecan-4 appears to be through its heparin-binding site as the chemotactic activity was inhibited by treating the cells with heparinase-1 and by blocking the pentasaccharide-binding site that mediates AT-III binding to HSPGs including Syndecan-4. Syndecan-4, a type 1 transmembrane receptor is atypical in that virtually all chemoattractant receptors are members of the PT inhibitable seven transmembrane class of G α i protein-linked receptors. An important question when new chemotactic activities are described is how potent (optimal concentration), effective (magnitude of effect) and specific is the mediator. In this paper the concentration of AT III is expressed as undefined units/mL (presumably related to its protease activity). This makes assessment of the potency of the chemotactic activity of AT-III in relation to physiologically relevant *in vivo* concentrations difficult to assess. Expressing the data as a ratio of the control makes it difficult to assess effectiveness especially when the distance travelled through the filter is used rather than the more usual way of expressing chemotactic activity as number of eosinophils per high power field migrating through the filter (which was used in the study by Furukawa et al. [35]). When expressed as cells/hpf an effective chemoattractant would be expected to produce a 10-fold or more enhancement over control rather than the 1.5-fold increase reported in this study. However the degree of migration was comparable with that seen for eotaxin and RANTES. AT-III is not selective for eosinophils as it is also active on neutrophils and mononuclear cells through binding to Syndecan-4 [36]. AT-III is part of the coagulation cascade. Expressed by the endothelium it binds and neutralizes thrombin. A potentially exciting aspect of this study is the possibility that AT-III could be playing a part in the activation step of eosinophil adhesion to endothelium and it would be very interesting to test this possibility in a flow-based adhesion assay. An unexpected and unexplained observation was the inhibition of chemotaxis to eotaxin and RANTES by pre-treatment with AT-III. While chemokines bind avidly to HSPG, which is thought to be relevant to the way in which they are presented to leucocytes by endothelium, they exert their chemotactic effects through well-defined G protein linked seven transmembrane receptors. Unlike neutrophils cross-desensitization by different classes of chemoattractants even when they are binding to 7TM receptors is not a particular feature of eosinophil responses [37]. This inhibition of chemotaxis may be more relevant to the anti-inflammatory properties of AT III [38]. Further investigation of this interesting observation is required. Chemoattractants usually have effects on leucocytes other than locomotion and it will be interesting to know whether AT-III also modulates eosinophil survival and mediator release especially as several signal transduction pathways appear to be involved in the chemotactic effects of AT-III/Syndecan-4.

Turning from a new chemotactic activity to a new source of well-established activity the paper by Furukawa et al. describes the generation of chemotactic activity by tracheal submucosal glands which is comprised of a combination of GM-CSF and RANTES. GM-CSF is a well-established growth factor for eosinophils, which primes eosinophils for chemotaxis and other functions and RANTES exerts its chemotactic effects on eosinophils predominantly through binding CCR3. Neither of these mediators are specific for eosinophils as GM-CSF is active on neutrophils and RANTES on lymphocytes and monocytes. In addition, large amounts of IL-8 a major neutrophil chemoattractant were generated by the submucosal cells in response to rhinovirus infection. These findings and similar findings in bronchial epithelial cells [39] emphasize the important contribution that structural cells make in directing the locomotion of leucocytes both into tissue and determining their fate thereafter. Although the authors suggest that chemotactic factors released by submucosal mucus glands may promote eosinophil transmigration through epithelium it is as likely that this would counteract gradients established by epithelial-derived mediators and be responsible for retaining eosinophils in tissue. They could also promote eosinophil interaction with submucosal glands possibly in turn enhancing mucus production, which is such an important feature of asthma and related diseases. This paper along with comparable studies in other cell types certainly offers a clear mechanism for the inflammatory effects of rhinovirus infection although this is often more neutrophilic than eosinophilic unless accompanied by allergen challenge [40]. It does not however address the question as to why asthmatics or patients with chronic obstructive pulmonary disease are more vulnerable to lower respiratory tract infection with respiratory viruses.

These papers contribute in a useful way to the extensive literature on mechanisms of eosinophil migration including a very large body of work on animal models of eosinophil migration, particularly the mouse asthma model, which I have hardly mentioned [41]. While the major pieces of the eosinophil migration jigsaw are in place many gaps remain to be filled. The complexity and redundancies involved in eosinophil tissue accumulation are considerable, which is one reason why we still do not know if blocking eosinophil accumulation in tissue will be of therapeutic benefit in asthma and related eosinophilic diseases [42].

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