

Matrix proteins and eosinophil mediator release

Eosinophils are thought to be important effector cells mediating the pathophysiological changes associated with asthma and other allergic diseases. Increased numbers of tissue eosinophils are a hallmark of these diseases which are characterized by increased amounts of eosinophil-derived mediators such as the eosinophil basic proteins, major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO), and lipid mediators such as the sulphidopeptide leukotrienes. In addition the array of cytokines generated by eosinophils including TGF α , and GM-CSF could have a range of effects such as tissue remodelling and leucocyte activation [1]. A striking feature of severe asthma, as well as some animal models of lung allergen challenge, is the dramatic degranulation of eosinophils to an extent that in its most florid form, intense staining for eosinophil granule proteins occurs in the absence of intact eosinophils [2].

For resting peripheral blood eosinophils to become fully competent for mediator release they need to be first primed. Priming agents include chemoattractants such as PAF, C5a and eotaxin signalling through G protein linked serpentine receptors and growth factors such as IL-5 and GM-CSF. Chemoattractants as well as causing chemotaxis, increase adhesiveness to endothelial and matrix ligands, cause shedding of L-selectin, reduce eosinophil density and enhance mediator release. Growth factors, while not themselves chemotactic, prime eosinophils for enhanced chemotactic responses and in addition to the changes mentioned above, delay eosinophil apoptosis and cause a marked and prolonged increase in expression of CD69, Mac-1, and ICAM-1 though *de novo* transcription, resulting in a receptor phenotype that mimics tissue eosinophils.

The most well characterized triggers for the generation and release of eosinophil mediators are engagement of Fc γ RII (CD32) and Fc α receptors particularly when IgG and IgA are presented bound to a nonphagocytosable adhesive surface such as a sepharose bead. If the eosinophils have been previously primed this can result in a marked release of eosinophil basic proteins [3]. Engagement of Fc ϵ R1, a receptor only very weakly expressed by eosinophils, or other putative IgE receptors, does not appear to result in significant mediator release [4]. To some extent the type of stimulus effects which mediator is generated. Cross-linking Fc receptors causes effective degranulation, but relatively modest release of lipid mediators compared to calcium ionophore which in turn does not cause much in the way of granule protein release. Both stimuli are good for superoxide release as is PMA which interestingly doesn't result in much release of either lipid mediators or granule

proteins (only 4% of ECP above background in the study by Xhu *et al.* reported in this issue of the journal) [5]. IFN γ does not result in superoxide release but can induce the synthesis and release of a number of cytokines [6]. Eosinophil mediator release can also be reliably induced by serum-opsonized particles such as yeast or sepharose beads which are thought to trigger release primarily through C3bi binding to Mac-1. The differences between priming and triggering stimuli are not absolute as chemoattractants and growth factors can also result in mediator release and effective triggering agents can cause priming. The difference between a priming event in which function is enhanced but no mediator release occurs and a triggering stimulus leading to mediator release is presumably due to different signal transduction pathways, the nature of which are still imperfectly understood.

In vivo tissue eosinophils are in contact with adhesive surfaces including matrix proteins and resident cells such as smooth muscle and the epithelium. In addition one of the most physiological *in vitro* models of eosinophil mediator release is during engagement with helminthic parasites where effective cytotoxicity is crucially dependent on adhesion to the larval tegument [7]. The manner in which eosinophils preferentially release their mediators onto a large surface suggests that adhesion, particularly through integrins, which have been shown in a wide variety of cell types to have a profound effect on cellular activation as a result of outside in signalling, could modulate eosinophil activation and mediator release [8]. Eosinophils express all four members of the CD18 leucocyte integrin family, LFA-1 (CD11a), Mac-1 (CD11b), CD11c and CD11d which can bind the endothelial/leucocyte-expressed ligands ICAM-1 (LFA-1, Mac-1), ICAM-2 (LFA-1), ICAM-3 (LFA-1) and VCAM-1 (CD11d), as well as a variety of matrix and serum proteins including albumin, fibrin, and fibrinogen (Fbg) through CD11b. In addition they express $\alpha 4\beta 1$ (VCAM-1 and fibronectin, [Fn]), $\alpha 4\beta 7$ (MAdCAM-1 and Fn) and $\alpha 6\beta 1$ (laminin) [9]. A number of groups have investigated how adhesion to surfaces, particularly Fbg and Fn may modulate eosinophil mediator release. The literature is complicated by the dependence of the outcome on the adhesive surface, the activating stimulus and the mediator measured. A number of studies, including the paper by Xhu *et al.* [5], have used f-mlp as a stimulus which has only a weak effect on unprimed eosinophils. It is however, a very effective stimulus for neutrophils so that contamination with neutrophils, which was common before the use of immunomagnetic selection, could effect the results [10]. The use of immunomagnetic selection to purify eosinophils brings

its own problems as the contaminating cell tends to be monocytes which readily generate mediators when in contact with matrix proteins so that contamination in the region of 0.5–1.0% can be an important confounding factor. Lastly eosinophils readily become activated when adhered to plastic surfaces even when coated with albumin, an effect mediated through CD18, the physiological relevance of which is unclear, but which makes it difficult to know what is the true background release. Taking these factors into account a consistent finding, also reported in the present study [5], is that although adhesion of eosinophils to matrix proteins doesn't directly cause mediator release (i.e. it is not a triggering stimulus), it can markedly prime eosinophils for enhanced release triggered by other agents. Thus when in suspension only PMA-induced eosinophils to generate superoxide, but a variety of agents were able to cause significant granule protein or superoxide release when eosinophils adhered to plastic surfaces uncoated or coated with albumin, fibrinogen and fibrin, an effect largely mediated through CD18 [11,12]. The data with fibronectin is less clear cut. We found that adhesion to fibronectin enhanced release of LTC₄ triggered by a suboptimal concentration of calcium ionophore [13]. We also found that adhesion to Fn caused release of GM-CSF leading to prolonged survival, although this was more clear cut with tissue rather than plasma Fn which contains more VLA-4 binding sites [14,15]. Similarly Neeley *et al.* found that adhesion to Fn enhanced EPO release triggered by f-mlp in the presence of cytochalasin B [16]. In each study the effect was inhibited by antibodies against $\alpha 4\beta 1$ or there was a contribution from $\alpha 4\beta 7$ was not studied. Xu *et al.* [5] found a trivial effect of adhesion to Fn on f-mlp-triggered release however, f-mlp stimulated less than 2% release of ECP. In this study adhesion to Fn did enhance PMA-induced release in the presence of Mn²⁺ which enhances integrin-mediated adhesion. However this effect was mediated through CD18 which was unexpected as CD18 integrins have not been previously shown to bind to Fn. In contrast Kita *et al.* [17], found that Fn inhibited eosinophil mediator release compared to plastic. The interpretation of this data depends on whether adhesion to either plastic or albumin which are active stimuli should be regarded as the correct baseline control or cells in suspension or plated on Teflon-coated surfaces. In summary therefore it is likely that *in vivo* eosinophils need to be adherent to a surface such as a matrix protein or resident tissue cell, for effective triggering of mediator release. This priming effect is the result of outside in signalling, largely through CD18 integrins, although $\alpha 4$ integrins and possibly $\alpha 6\beta 1$ may also be involved, depending on the nature of the surface and the trigger involved.

In the light of these findings what are likely to be the triggers for eosinophil degranulation in allergic disease?

There is limited evidence that asthma, rhinitis and atopic dermatitis are associated with autoantibodies, so it is difficult to envisage Fc-mediated triggering as being involved. Similarly complement activation is not a feature of allergic disease. It is possible that eosinophil mediator release is due to the synergistic effect of growth factors, chemoattractants and matrix protein/integrin-mediated interactions in the airways. This is particularly likely to be the case in milder disease characterized by piecemeal degranulation as oppose to the more explosive degranulation seen in severe eosinophilic inflammation [18]. In these circumstances it remains possible that other, as yet to be described, triggers of eosinophil mediator release are involved.

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A. J. WARDLAW
Institute for Lung Health
Leicester University
Glenfield Hospital
Groby Road
Leicester
LE3 9QP
UK