

EDITORIAL

IL-5 priming of eosinophil function in asthma

Eosinophils were once thought to help to resolve acute asthma by scavenging allergens and cellular debris in the airway, but their prevalence in the airways and blood of persistent asthmatics and their ability to produce toxic granule proteins, pro-inflammatory cytokines, oxygen radicals and lipid mediators incriminates them as principal culprits of airway inflammation in asthma. Eosinophils consistently appear in the inflamed asthmatic airway. In atopic subjects, influx of eosinophils into BAL fluid is apparent during and following the late response to inhaled allergen challenge, at a time when bronchial responsiveness is enhanced. In numerous studies, eosinophilia has been demonstrated in induced sputum, BAL fluid, bronchial biopsies and blood of both allergic and non-allergic patients with clinical asthma [1]. Eosinophil-derived basic proteins are over-expressed in lung tissue and BAL fluid from asthmatics, suggesting that eosinophils are both over-represented and activated [2]. Enhanced numbers of eosinophils in biopsies and BAL fluid correlate with asthma symptom scores, impaired lung function (FEV₁), and non-specific BHR, causally linking eosinophils to asthma severity [1].

Eosinophils proliferate and differentiate within the bone marrow under the influence of cytokines including IL-3, IL-5 and GM-CSF, with IL-5 having the most cell-specific effects on terminal eosinophil maturation. The importance of IL-5 in inducing airway eosinophilia and bronchial hyperresponsiveness has been demonstrated in murine models of asthma, although complex interactions with other cytokines are also involved [3]. In asthmatics, IL-5 inhalation increases eosinophil counts and ECP levels in induced sputum, with a modest increase in methacholine bronchial responsiveness [4]. Expression of IL-5 mRNA in bronchial biopsies correlates highly significantly with asthma severity, baseline lung function (PEF and FEV₁), and bronchial responsiveness to histamine [5].

Eosinophils are equipped with a powerful armoury of pre-formed and newly synthesized mediators capable of inducing the classical pathophysiological features of asthma, including bronchoconstriction, airway oedema, mucus hypersecretion, epithelial damage, airway remodeling, and BHR. Among the lipid mediators, eosinophils produce the potent bronchoconstrictor LTC₄ in amounts comparable to mast cells and basophils, as well as 15-lipoxygenase products (15-HETE, lipoxins), modified

phospholipids (PAF) and prostanoids (TXA₂, PGE₂). Protein mediators include a wide range of cytokines and chemokines, particularly IL-3, IL-5, GM-CSF, eotaxin and RANTES, suggesting that eosinophil migration and activity may be sustained by autocrine mechanisms, while synthesis of IL-1, IL-4 and IL-16 suggests immunomodulatory activity on other cells. The eosinophil specific granule contains major basic protein (MBP) in its core, and ECP, EPO and EDN in its matrix; these granule-derived proteins have cytotoxic activity for helminths and are highly toxic to bronchial epithelium [2]. Eosinophils stimulated to undergo the respiratory burst produce large amounts of oxygen metabolites including superoxide (O₂⁻) and hydrogen peroxide (H₂O₂) that also have cytotoxic activity for multicellular parasites and for host cells.

From the circulation, eosinophils interact with endothelial cells that express adhesion molecules, including VCAM-1, which recognizes VLA-4 found specifically on eosinophils but not neutrophils, and ICAM-1 that binds CD11/CD18 molecules on a range of leucocyte types including eosinophils. Eosinophil transmigration across HUVECs appears to depend primarily on CD11/CD18 not VLA-4, but upregulation of endothelial VCAM-1 by IL-4 and other factors may make the VCAM-1/VLA-4 interaction more important in selective eosinophil recruitment *in vivo*. Interaction with endothelial cells via these adhesion molecules activates eosinophils to generate LTC₄, granule proteins and superoxide [6]. Within the airway mucosa, directed migration and activation of eosinophils may depend further on receptor-dependent interactions with the local mediator environment. Eosinophils express receptors for lipids (PAF, LTB₄, LTD₄), complement components (C3a, C5a), bacterial peptides (FMLP), chemokines (IL-8, RANTES, eotaxin, MCP-1, MCP-3), cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-9, IL-16, GM-CSF, IFN γ , and TNF α) and immunoglobulins (IgA, IgG, IgE) [7]. Thus, eosinophils are equipped with a range of molecular interfaces to provide access to the airway and to activate inflammatory mediator release.

The influx of eosinophils into the asthmatic airway is not an opportunistic response to local factors of a uniform population of end-stage cells, but may be predetermined by factors acting within the bone-marrow and within the circulation [8] to produce a sub-population of hyper-responsive or 'primed' cells. These cells may be presumed

to be prepared for an enhanced role in airway inflammatory processes. The term priming indicates the way in which factors that do not ostensibly activate eosinophils, or that activate them only at high concentrations, can produce a covert enhancement of their potential for adhesion, migration and mediator synthesis that is revealed only in response to subsequent stimuli. Many phenotypic and functional anomalies are observed in eosinophils from allergic and asthmatic patients, and these can usually be replicated *in vitro* by treating normal blood eosinophils with priming factors. Priming factors for eosinophils include neuropeptides (CGRP, substance P) and PAF, but the most important are eosinophilopoietic cytokines, of which GM-CSF is the most potent and IL-5 the most eosinophil-specific.

Among the first evidence suggesting a primed eosinophil phenotype in allergic disease was the finding of a higher proportion of low-density blood eosinophils than in normal subjects. Such hypodense eosinophils have a higher cell volume and a lower density of granule proteins than normodense cells, and are associated with an increased capacity for LTC₄ release and enhanced helminthotoxicity. Hypodense eosinophils were hypothesized to represent a population of relatively immature cells newly released from bone marrow, but are more likely to be a population of activated cells that have been primed by cytokines and other factors. Indeed, *in vitro*, normal eosinophils are converted to a hypodense phenotype by IL-5 and related cytokines and by PAF, associated with enhanced LTC₄ synthesis [9].

Eosinophils isolated from the blood, BAL and sputum of allergic and asthmatic patients show a number of other phenotypic changes that reveal *in vivo* priming. Blood eosinophils from allergic subjects have an enhanced ability to transmigrate across endothelial cells *in vitro*, and this involves *in vivo* priming by IL-5 and related cytokines [10]. They express significant quantities of the early activation marker CD69, the β_2 integrin CD11b, and the intercellular adhesion molecule ICAM-1, which are expressed in very low or negligible amounts on normal eosinophils. Following allergen challenge, BAL fluid eosinophils further increase their expression of CD11b and other activation markers [11].

Eosinophils from allergic patients show enhanced chemotactic responses to fMLP and PAF *in vitro* compared to normal cells, and inhaled allergen challenge further enhances chemotactic responses to IL-8 [12]. In normal eosinophils, IL-5 enhances chemotactic responses to a range of chemotaxins, but those from patients with asthma and eosinophilia are unaffected [13], confirming that prior IL-5 priming occurred *in vivo*. Blood eosinophils from asthmatics generate more ECP and EPX following serum-opsonized Sephadex stimulation than normal eosinophils,

and serum levels of these eosinophil-derived granule proteins are also higher in asthmatics [14]. GM-CSF, IL-3 and IL-5 greatly enhance the interaction of human eosinophils with opsonized particles by changing the affinity of complement receptor type 3 [15]. These data taken together with other studies show that eosinophils are susceptible to phenotypic modification by circulating and local factors that enhance their capacity to respond to inflammatory stimuli by adhesion, diapedesis, migration and mediator release.

The mechanisms of these priming processes remain unclear. The paper in the current issue of *Clinical and Experimental Allergy* by Dr Woschnagg and colleagues from Uppsala, Gothenberg and Trieste [16] expands our understanding of eosinophil priming by showing that the production of cytotoxic oxygen radicals by eosinophils from allergic patients is primed by natural exposure to a seasonal aeroallergen, and by delineating the signal transduction processes involved when IL-5 further primes oxidative metabolism *in vitro*. The researchers have pioneered the study of seasonal aeroallergen (birch pollen) exposure as a paradigm of 'real-life' asthma and allergic rhinitis. Inflammatory changes in the airways and blood examined before and during natural exposure to an environmental allergen provide a more accurate picture of the natural history of allergic disease than laboratory studies in which patients are exposed to a single dose of inhaled allergen. Such laboratory challenges use allergen doses that are relatively enormous compared to the sustained low-level allergen exposure that drives allergic disease at home or at work. The high prevalence of sensitivity to birch pollen in allergic patients in southern Sweden, and the predictable onset of the birch pollen season at the beginning of May each year, are further practical advantages of this approach. By selecting patients without concurrent sensitivity to perennial allergens such as house dust mites, the investigators are able to maximize the power of such studies to distinguish between the baseline and allergen-driven disease states.

The authors have previously shown that in birch-sensitive patients, the birch pollen season is accompanied by significant increases in eosinophils in BAL fluid and in bronchial biopsies [17,18]. They have also shown that blood eosinophils are primed for increased adhesion *in vitro* to VCAM-1 and ICAM-1 during the pollen season [19], and that subsequent migratory responses to RANTES and IL-8 depend on prior priming by IL-5 [20]. Increased eosinophil chemoattractant activity in asthmatic BAL fluid during the birch pollen season was ascribed to IL-5 and RANTES [21]. The seasonal allergen approach is therefore well-suited to investigations of *in situ* allergen-driven priming of blood eosinophils by IL-5.

In the present study [16], Woschnagg and colleagues

immunomagnetically purified blood eosinophils from allergic patients with birch pollen seasonal rhinitis with or without asthma, and from normal subjects, and cells were stimulated with PMA, which activates protein kinase C (PKC). Oxidative metabolism was assessed by a lucigenin enhanced chemiluminescence technique. The initial rate of PMA-stimulated oxidative metabolism was higher (lower initial $T_{1/2}$) in the allergic rhinitis patients in the pollen season than in the normal controls, while the two groups showed no differences in oxidative metabolism pre-season. Priming of the response in the allergic patients, appeared to be particularly marked in the subgroup of patients with concomitant asthma.

On closer inspection, the result is problematical in that the group difference appears to be due to a *reduction* in oxidative metabolism in the control subjects in-season, rather than a real increase in the allergic patients. The problem is compounded by the discrepancy with previous data from the same researchers that suggested a reduced oxidative metabolism in-season in *allergic* patients compared to pre-season values and to in-season controls [22]. The authors suggest that the increased proportion of hypodense eosinophils in allergic patients may account for the anomalies in the earlier study, but data from the present work demonstrate that the rise in $T_{1/2}$ following PMA is independent of eosinophil density. As the data imply, the issue of hypodensity may be a red herring. Indeed, the increased proportion of hypodense eosinophils in allergic subjects *per se* has been suggested to be an artefact of isolation by density gradient centrifugation techniques, but one that nevertheless reflects an underlying priming process in eosinophils from allergic subjects that is better marked by enhanced LTC₄ synthesis [23]. The discrepancy may instead be related to the use of the initial rate of oxidative metabolism (measured as the $T_{1/2}$ of the rise following PMA stimulation) as the primary outcome measure, when the maximal degree of oxidative metabolism showed contradictory outcomes in a number of aspects, including a reduced maximal response following IL-5 treatment. Analysis of the area under the curves to assess total oxidative metabolism over time may resolve these contradictions and provide a more physiologically relevant measure of this model to assess oxidative damage to host tissues *in vivo*.

Despite these difficulties in interpretation, the study by Woschnagg and colleagues [16] provides intriguing information on the mechanism of IL-5 priming. Irrespective of baseline comparisons between the groups, IL-5 treatment *in vitro* increased the initial rate of oxidative metabolism (reduced the $T_{1/2}$ rise) in both allergic and normal subjects in-season and pre-season. This is perhaps surprising, as previous studies suggest that IL-5 is critical in priming chemotactic and other responses of eosinophils

in vivo; eosinophils from allergic subjects therefore fail to show further priming following IL-5 treatment, while unprimed eosinophils from normal subjects display a clear response [13]. The authors interpret their data to suggest that priming of eosinophil oxidative metabolism *in vivo* in allergic subjects is due to factors other than IL-5, but the study does not indicate what such factors might be.

Analysis of the signal transduction mechanisms underlying IL-5 priming was performed using selective inhibitors. The IL-5 receptor is a heterodimeric membrane protein with an IL-5-specific 60–80 kDa α -subunit, and a 120-kDa β -subunit common to the receptors for IL-3 and GM-CSF. Binding of IL-5 causes activation of numerous tyrosine kinases including *lyn*, *yes*, *blk*, *tec* and JAK2, leading to tyrosine phosphorylation of multiple signal transduction pathways. These include: the JAK2-Stat1 pathway that regulates gene transcription; the p21 ras/Raf-1/MEK/MAP kinase pathway that regulates gene transcription and also primes LTC₄ synthesis by phosphorylating phospholipase A₂; and the phosphatidylinositol 3-kinase (PI3K) pathway which is implicated in actin rearrangement and cell migration [24].

Woschnagg and colleagues show firstly that three inhibitors of PKC, namely staurosporine, Ro 318220, and Go 6938, substantially inhibited PMA-stimulated oxidative metabolism. This is predictable based on the ability of PMA and other phorbol esters to induce oxidative metabolism by activating PKC. The degree of inhibition of total oxidative metabolism by staurosporine was so marked that it masked any realistic analysis of the effect of IL-5 priming on the initial rise in radical formation. Secondly, it was shown that wortmannin, an inhibitor of PI3K, slowed the initial rate of oxidative metabolism, but had no effect on maximal radical production, and that the inhibition could not be surmounted by IL-5. This may indicate that PI3K is involved in the IL-5 priming of the initial rise in oxidative metabolism. The observation implies dual involvement of PI3K in responses to cytokines such as IL-5 (acting at tyrosine kinase-dependent receptors) and to direct chemoattractants such as PAF, fMLP and LTB₄ that act via G-protein-linked 7-transmembrane receptors. Such cross-talk may underlie the synergism between IL-5 and direct chemoattractants in priming eosinophil migration [24].

The MEK (Erk kinase) inhibitor PD 98059 reduced both the initial rate and the maximal rate of oxidative metabolism, and this effect was partially reversed by IL-5 treatment, indicating that IL-5 may partially bypass MEK and directly activate a site further downstream in the pathway. The authors postulate that this site may be Erk-1 and Erk-2 which have previously been shown to mediate IL-5-induced priming of LTC₄ synthesis in response to stimulation of eosinophils with fMLP [25]. Activation of

Erk-1/2 is also thought to activate the NADPH oxidase involved in production of oxygen radicals. Thus, in an analogous manner, IL-5 may prime NADPH oxidase activity in eosinophils by directly activating Erk-1/2, such that a greater oxidative metabolism is observed when PMA indirectly activates Erk-1/2 via PKC.

Investigation of the signal transduction mechanisms of cytokine priming is at an early stage, despite rapid advances following the development of immunomagnetic techniques to isolate primary eosinophils from blood. Studies have shown that suppression of eosinophil apoptosis by IL-5 requires *lyn*, JAK2, and Raf-1 kinase activity, while priming of ECP release and of CD11b-dependent adhesion requires only the Raf-1 kinase activity [26]. This demonstrates some specificity of signalling pathways for distinct eosinophil functions, which stands in contrast to the overlapping ability of several members of the IL-5 cytokine family to prime eosinophils. Studies such as that by Woschnagg and colleagues may point the way towards novel therapeutic strategies to prevent priming of abnormal eosinophil function in asthma and allied inflammatory disease. The need for such a strategy is highlighted by the recent studies showing that humanized monoclonal antibodies directed against IL-5 fail to block the early or late bronchoconstrictor responses to allergen in atopic asthmatics, despite significant reductions in eosinophil counts in the circulation and in sputum [27]. The failure of anti-IL-5 may be due to the ability of a number of related cytokines to prime the function of residual eosinophil subpopulations via their common receptor β -subunits. Inhibition of shared signalling mechanisms may block eosinophil activation more effectively.

References

- Robinson DS. Eosinophils and asthma: what do measurements tell us? *Clin Exp Allergy* 1995; 25:795–8.
- Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000; 105:651–63.
- Foster PS. Allergic networks regulating eosinophilia. *Am J Respir Cell Mol Biol* 1999; 21:451–4.
- Shi HZ, Xiao CQ, Zhong D et al. Effect of inhaled interleukin-5 on airway hyperreactivity and eosinophilia in asthmatics. *Am J Respir Crit Care Med* 1998; 157:204–9.
- Humbert M, Corrigan CJ, Kimmitt P et al. Relationship between IL-4 and IL-5 mRNA expression and disease severity in atopic asthma. *Am J Respir Crit Care Med* 1997; 156:704–8.
- Munoz NM, Hamann KJ, Rabe KF et al. Augmentation of eosinophil degranulation and LTC₄ secretion by integrin-mediated endothelial cell adhesion. *Am J Physiol* 1999; 277:L802–L810.
- Weller PF. Human eosinophils. *J Allergy Clin Immunol* 1997; 100(3):283–7.
- Corrigan CJ, Haczku A, Gemou-Engesaeth V et al. CD4 T-lymphocyte activation in asthma is accompanied by increased serum concentrations of interleukin-5. *Am Rev Respir Dis* 1993; 147:540–7.
- Rothenberg ME, Petersen J, Stevens RL et al. IL-5-dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability, accelerated hypodensity, and sustained antibody-dependent cytotoxicity. *J Immunol* 1989; 143:2311–6.
- Moser R, Fehr J, Olgiati L et al. Migration of primed human eosinophils across cytokine-activated endothelial cell monolayers. *Blood* 1992; (79):2937–45.
- Busse WW, Nagata M, Sedgwick JB. Characteristics of airway eosinophils. *Eur Respir J (Suppl.)* 1996; 22:132s–135s.
- Warringa RA, Mengelers HJ, Raaijmakers JA et al. Upregulation of formyl-peptide and interleukin-8-induced eosinophil chemotaxis in patients with allergic asthma. *J Allergy Clin Immunol* 1993; 91:1198–205.
- Sehmi R, Wardlaw AJ, Cromwell O et al. Interleukin-5 selectively enhances the chemotactic response of eosinophils obtained from normal but not eosinophilic subjects. *Blood* 1992; 79:2952–9.
- Carlson M, Hakansson L, Peterson C et al. Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. *J Allergy Clin Immunol* 1992; 87:27–33.
- Blom M, Tool AT, Kok PT et al. Granulocyte-macrophage colony-stimulating factor, interleukin-3 (IL-3), and IL-5 greatly enhance the interaction of human eosinophils with opsonized particles by changing the affinity of complement receptor type 3. *Blood* 1994; 83:2978–84.
- Woschnagg C, Garcia R, Rak S et al. IL-5 priming of the PMA-induced oxidative metabolism of human eosinophils from allergic and normal subjects during a pollen season. *Clin Exp Allergy* 2001; 31:555–64.
- Rak S, Björnson A, Håkansson L et al. The effect of immunotherapy on eosinophil accumulation and production of eosinophil chemotactic activity in the lung of subjects with asthma during natural pollen exposure. *J Allergy Clin Immunol* 1991; 88:878–88.
- Seymour ML, Aberg D, Riise G et al. Seasonal allergen exposure increases expression of leukotriene pathway enzymes and induces eosinophil influx in bronchial mucosa of atopic asthmatics. *J Allergy Clin Immunol* 1998; 101:711(abstract).
- Hakansson L, Heinrich C, Rak S et al. Priming of eosinophil adhesion in patients with birch pollen allergy during pollen season: effect of immunotherapy. *J Allergy Clin Immunol* 1997; 99:551–62.
- Lampinen M, Rak S, Venge P. The role of interleukin-5, interleukin-8 and RANTES in the chemotactic attraction of eosinophils to the allergic lung. *Clin Exp Allergy* 1999; 29:314–22.
- Venge J, Lampinen M, Hakansson L et al. Identification of IL-5 and RANTES as the major eosinophil chemoattractants in the asthmatic lung. *J Allergy Clin Immunol* 1996; 97:1110–5.
- Woschnagg C, Rak S, Venge P. Oxygen radical production by blood eosinophils is reduced during birch pollen season in allergic patients. *Clin Exp Allergy* 1996; 26:1064–72.
- Brujinzeel PL, Virchow JCJ, Rihs S et al. Lack of increased

- numbers of low-density eosinophils in the circulation of asthmatic individuals. *Clin Exp Allergy* 1993; 23:261–9.
- 24 Koenderman L, Van der Bruggen T, Schweizer RC et al. Eosinophil priming by cytokines: from cellular signal to *in vivo* modulation. *Eur Respir J* 1996; 9:S119–S125.
 - 25 Bates ME, Green VL, Bertics PJ. ERK1 and ERK2 activation by chemotactic factors in human eosinophils is IL-5-dependent and contributes to LTC₄ synthesis. *J Biol Chem* 2000; 275:10968–75.
 - 26 Pazdrak K, Olszewska-Pazdrak B, Stafford S et al. Lyn, Jak2, and Raf-1 kinases are critical for the antiapoptotic effect of interleukin-5, whereas only Raf-1 kinase is essential for eosinophil activation and degranulation. *J Exp Med* 1998; 188:421–9.
 - 27 Leckie MJ, Ten Brinke A, Lordan J et al. SB240563, a humanized anti-IL-5 monoclonal antibody: initial single-dose safety and activity in patients with asthma. *Am J Respir Crit Care Med* 1999; 159(3):A624(Abstract).

A.P. SAMPSON

Division of Respiratory Cell and Molecular Biology

Level F South Block (825)

Southampton General Hospital

Tremona Road

Southampton SO16 6YD

UK

E-mail: aps@soton.ac.uk