

The role of the eosinophil in the pathogenesis of asthma

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Introduction

Eosinophils were first clearly identified by Ehrlich in 1879 (56) and the association of increased numbers of blood eosinophils with asthma was soon recognized (33). It is now well established that large numbers of eosinophils, together with mononuclear cells, are frequently present in and around the bronchi of patients who have died from asthma (31). Charcot-Leyden-crystals (CLC) are another well-known eosinophil associated feature of the asthma process, which has now been identified as membrane-derived lysophospholipase (118). There has been a continuing debate regarding the role of eosinophils in asthma. In short, do they ameliorate the inflammatory process or promote it (117)? On the one hand eosinophil-derived products biodegrade mediators such as the peptide leukotrienes, which comprise the slow-reacting substance of anaphylaxis (leukotriene (LT) C₄, D₄, E₄). This is achieved through both oxidation to their respective sulphoxides and by conversion to the 6-trans diastereoisomers of leukotriene (LT) B₄ by granule-derived eosinophil peroxidase (54, 119). The original observation that eosinophil-derived arylsulphatase could deactivate SRS-A was probably due to biodegradation of LTC₄ to LTE₄ by contaminating dipeptidases (73, 114). Similarly eosinophils also contain histaminase located in the granule compartment

which oxidizes histamine to its catabolite imidazole acetic acid (121). However, in recent years increased understanding of the biology of the eosinophil, particularly with regard to their granule and membrane-derived products, has emphasized a pro-inflammatory rather than homeostatic role for this cell type. Many investigators now believe that eosinophils are a major effector of tissue damage in asthma (63). Several recent clinical studies, particularly those involving bronchoalveolar lavage, have provided more direct evidence to support this concept.

The purpose of this review is to examine some recent findings on the biology of the eosinophil which might be relevant to the role of this cell in asthma and to evaluate various clinical studies which have identified an association between eosinophils, the late-phase response to antigen challenge, non-specific bronchial hyperreactivity and day-to-day asthma. All the work referred to has been performed on human eosinophils unless otherwise stated.

EOSINOPHIL BIOLOGY

Eosinophil heterogeneity

Techniques developed to separate leucocytes according to their density (106) revealed that eosinophil populations are often heterogeneous.

“Normal” density cells are found in subjects with and without an eosinophilia, whereas “light density” eosinophils are usually present in subjects with an eosinophilia in association with a variety of causes including asthma (39). The light density eosinophils are functionally and morphologically distinct from the normal density cells and are generally regarded as being more “activated” (101). They can, for example, kill parasite targets more effectively (90) and produce more LTC₄ (97). It is not known whether the light density cells are derived from a separate population of bone marrow precursor cells or whether they are activated by the disease process. The latter appears more likely since activation of normal density cells by various mediators, such as f-Met-Leu-Phe, the ECF-A tetrapeptides and PAF-acether, can result in many of the features of light density cells, such as enhanced cytotoxicity (4), and receptor expression (66). It is of interest that tissue eosinophils are of light density, at least in some diseases (102), and eosinophils in lung lavage in asthma can have the vacuolated appearance typical of activated eosinophils (Wardlaw, A, personal observation).

Eosinophil secretion

Relatively little is known about the mechanisms and stimuli which lead to eosinophil degranulation, particularly at an intracellular level (101). Triggering of membrane components, such as IgG (Fc) and complement (C3b) receptors, leads to release of both granule and membrane derived mediators. Physiological models of secretion suggest that the eosinophil needs to become attached to an appropriately opsonized surface, such as IgG coated Sepharose beads, and releases its mediators as part of a process similar to “frustrated phagocytosis” described for neutrophils. This mechanism is best illustrated in the way the eosinophil kills parasite targets (76).

Mediators

The mediators of eosinophil leucocytes, like those of the mast cell, can be conveniently divided into granule-associated and membrane-derived (Fig. 1). The membrane-derived mediators consist principally of eicosanoids derived from activation of the 5 and 15 lipoxygenase pathways and PAF-

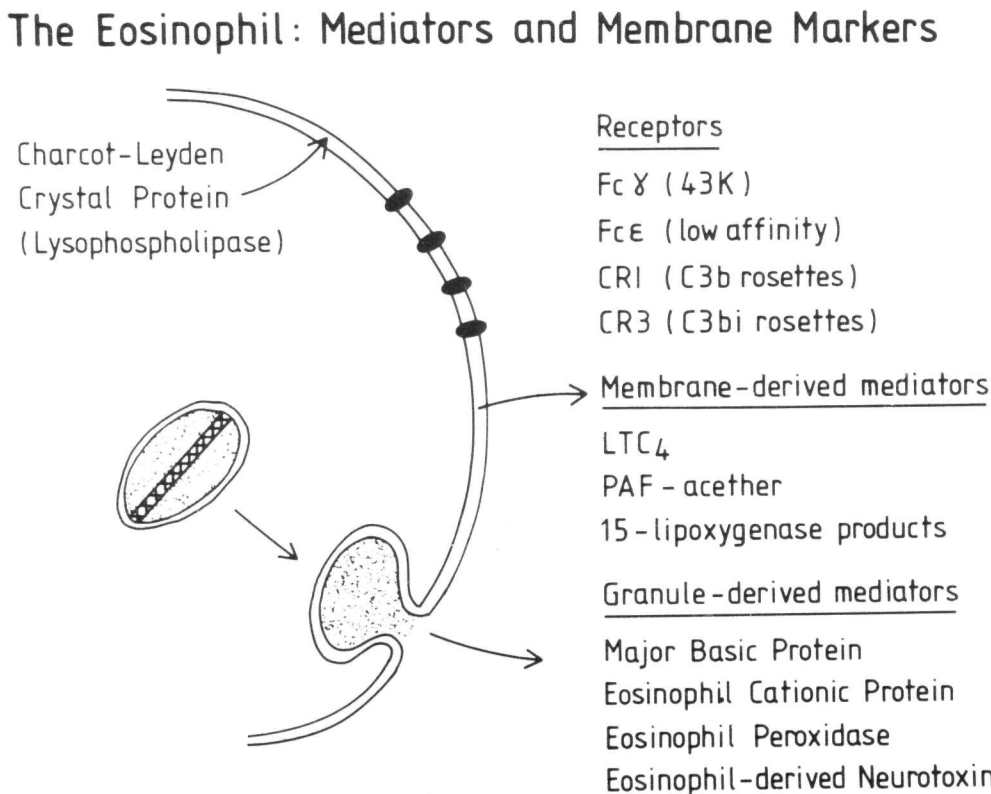


Fig. 1. Diagram to illustrate eosinophil-derived mediators and membrane receptors.

acether (1-0-alkyl-2-acetyl sn-glycerol phosphocholine). The Charcot-Leyden-crystal (CLC) is also membrane-derived (120). It has no definite biological function although it could be involved in reacylation of lysophospholipids which are generated as a result of phospholipase A₂ activity and have a detergent effect on the cell membrane in high concentrations. Basophils also generate CLC protein in similar amounts to eosinophils (2). However, basophils have not been identified in asthmatic airways and it is unlikely that they are the source of CLC protein in asthmatic sputum. Several investigators have demonstrated that eosinophils can generate relatively large amounts of LTC₄ (5S-hydroxy-6R-S-glutathionyl-7,9-trans-11,14-cis-eicosatetraenoic acid) after stimulation with the calcium ionophore A23187 (98, 119) and these cells appear to be potentially able to produce more of this potent spasmogenic mediator than most other cell types. For instance after stimulation with the calcium ionophore A23187, normal density eosinophils maximally generate 40 ng/10⁶ cells of LTC₄ and light density eosinophils 70ng/10⁶ cells, whereas neutrophils, monocytes, alveolar macrophages

and mast cells generate 7, 30, 10 and 25 ng/10⁶ cells respectively. In contrast, eosinophils produce only 6ng/10⁶ cells of the chemotatic mediator LTB₄ (5S-12R-dihydroxy-6,14-cis-8,10-trans-eicosatetraenoic acid), which is also derived from arachidonic acid via the 5 lipoxygenase pathway (compared to neutrophils, monocytes and macrophages which can generate 50, 70 and 200 ng/10⁶ cells of LTB₄ respectively) (73). Eosinophils can also produce substantial quantities of 15-HETE via the 15 lipoxygenase pathway; however, this has only been shown to occur after calcium ionophore stimulation in the presence of exogenously added arachidonic acid (53). A more physiological stimulus for the release of LTC₄ using IgG-coated Sepharose beads has recently been described (97). Using this technique it was shown that light density eosinophils generate more LTC₄ than normal density eosinophils and that normal density eosinophils activated by the synthetic chemotactic peptide f-Met-Leu-Phe generate increased amounts of this mediator. In addition, it was shown that a factor produced by unstimulated monocytes cultured for about 18 h from patients

Table 1
Properties of principal eosinophil granule proteins

Name	Molecular weight (x 10 ⁻³)	pI	Activities	Site
MBP	10 (man) 11 (guinea pig)	> 11	1) Toxic to parasites, murine tumour cells, many mammalian cells; 2) causes histamine release from basophils and rat mast cells; 3) neutralizes heparin	core
ECP	21	> 11	1) Shortens coagulation time, alters fibrinolysis; 2) toxic to parasites; 3) potent neurotoxic; 4) inhibits cultures of peripheral blood lymphocytes; 5) causes histamine release from mast cells.	matrix
EDN	17.9	basic	Potent neurotoxin.	matrix
EXP	18	basic	1) Neurotoxin; 2) inhibits cultures of peripheral blood lymphocytes; 3) toxic to parasites.	matrix
EPO	71-77 (man) 75 (horse)	> 11	In the presence of H ₂ O ₂ + halide 1) kills microorganisms and tumour cells; 2) initiates mast cell secretion; 3) inactivates leukotrienes; 4) causes histamine release from rat mast cells.	matrix

(From Gleich & Adolphson, ref. 45)

with a peripheral blood eosinophilia, when pre-incubated with normodense eosinophils for 10 min caused enhancement of LTC_4 production by IgG-Sepharose beads in a time and dose dependent fashion (36). A modification of this technique using Sepharose beads coated with antibody-antigen complexes prepared from extract of *Aspergillus fumigatus* and IgG fraction of serum from patients with allergic bronchopulmonary aspergillosis (ABPA) also resulted in the release of LTC_4 (68). This possibly mimics the *in vivo* situation where the fungal hyphae and specific IgG antibody coexist and are closely associated with the airway epithelium which, like the Sepharose beads, provides a non-phagocytosable surface. Approximately 25% of the maximal release obtained by calcium ionophore can be generated by these complexes.

PAF-acether is also a potent mediator of inflammation which has several biological activities relevant to asthma. These include granulocyte and platelet activation, *in vitro* and bronchoconstriction and induction of prolonged bronchial hyperreactivity after inhalation (24, 77, 96). Although PAF-acether is generated by several cell types including neutrophils, macrophages, platelets and possibly mast cells (87), it has also been shown to be released in considerable amounts (100 ng/ 10^6 cells) by light density eosinophils following stimulation with calcium ionophore (61). Increased acetyl transferase activity (the enzyme which acetylates lyso-PAF to produce PAF-acether) has also been identified following stimulation of normal density eosinophils with calcium ionophore, although only picogram quantities of PAF-acether were generated (72). Whether PAF-acether is produced by eosinophils following a physiological stimulus such as the IgG-Sepharose bead model described above is still not known.

The eosinophil granule contains several basic proteins which have been shown to be cytotoxic to several cell types (Table 1). Many of the studies with direct relevance to asthma have involved major basic protein (MBP) which has a molecular weight of 10,000 Da. It is contained predominantly in the core of granule, and in the guinea pig it accounts for 50% of the total granule protein (42, 43). It is also present within

basophils, but only in very small amounts (1). MBP was shown to be cytotoxic for schistosomula of *Schistosoma mansoni*, and eosinophils released MBP when in contact with IgG-coated schistosomula detected both by immunofluorescence of the parasite and radioimmunoassay of the culture supernatants (12). MBP is also toxic for several other parasites including newborn larvae of *Trichenella spiralis* and trypomastogote stage of *Trypanosoma cruzi* (70, 115). Of particular interest is the ability of MBP to cause damage and in higher concentrations complete destruction of respiratory epithelium *in vitro* in both guinea pigs and humans (38, 44), mimicking the epithelial damage characteristic of asthma (31). MBP causes non-cytotoxic release of histamine from basophils (86) but this has not been confirmed for human lung mast cells.

Eosinophil receptors

Eosinophils possess complement receptors (CR1 and CR3) and IgG (Fc) receptors (3, 35). Recently, eosinophils have been shown to possess IgE (Fc) receptors both by the rosette technique (IgE myeloma protein covalently linked to red cells), and radioligand binding studies using ^{125}I labelled IgE (14, 110). This receptor appears to be similar to the IgE receptors on platelets and macrophages (15). Although the IgE receptor on the eosinophil ($\text{Fc}_\epsilon\text{R}_2$) is of considerably lower affinity than the IgE receptor on mast cells and basophils (79) it is of similar affinity to the eosinophil IgG (Fc) receptor. Eosinophil IgE (Fc) receptors appear to be more fully expressed on both hypodense and normodense eosinophils from patients with an eosinophilia than on eosinophils from normal subjects. This is not the case for IgG receptors, suggesting that the IgE (Fc) receptor is more closely associated with activation of the eosinophil (13).

The functional properties of the IgE (Fc) receptor have not been fully elucidated. IgE (Fc) dependent killing of schistosomula of *S. mansoni* by eosinophils has been demonstrated (18). A monoclonal antibody (BB10) against the eosinophil IgE (Fc) receptor was prepared by immunization with hypodense eosinophils. This antibody inhibited IgE dependent, but not IgG dependent, cytotox-

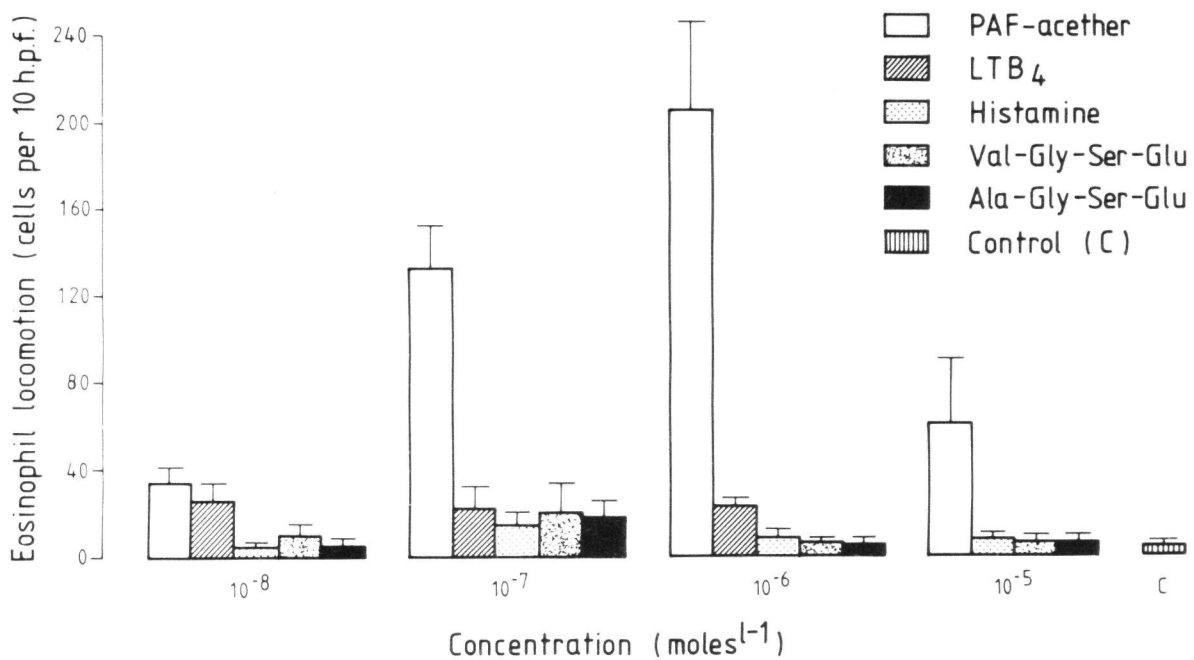


Fig. 2. Eosinophil locomotion to PAF-acether and other chemically characterized chemoattractants.

icity and IgE rosette formation. It also cross reacted with the IgE receptor on platelets and monocytes demonstrating a common antigenicity between these receptors. Cross reactivity with the mast cell IgE receptor was not observed (16). IgE dependent killing is also inhibited by an antibody specific for CR3 but not by an antibody against CR1. The BB10 antibody does not inhibit CR3 rosette formation, suggesting that there is no cross reactivity between BB10 and CR3 receptor. Incubation of light density eosinophils with anti-IgE also resulted in release of eosinophil peroxidase as measured by chemiluminescence and MBP (13). Only the IgE receptor on light density eosinophils appears to be functionally active, possibly due to increased IgE (Fc) receptor expression (18). Tissue eosinophils (e.g. obtained from lung lavage in patients with pulmonary eosinophilia) and blood eosinophils from subjects with high levels of IgE show receptor occupancy by IgE, raising the possibility of direct interaction between the eosinophil and inhaled antigen in the airways. IgG receptors do not appear to be occupied by cytophilic antibody in the same way (17, 102).

Eosinophil chemotaxis

It is still unclear as to which mediators are involved in attracting eosinophils into the bron-

chi either in models of asthma such as allergen challenge or in day-to-day asthma. Many factors and mediators with eosinophilotactic activity have been described (63). For instance, supernatants from chopped lung challenged with allergen or anti-IgE were found to have eosinophil chemotactic activity. This activity lay in the low molecular weight region (360–1000 Da) and was termed the eosinophil chemotactic factor of anaphylaxis (ECF-A) to distinguish it from other eosinophil chemotactic factors, such as those derived from the complement system (ECF-C) (64). The characteristics of ECF-A generation in terms of IgE dependent stimulation, time course and biochemical requirements for release are similar to histamine and suggest that ECF-A is a mast cell-derived or at least mast cell-associated mediator. This was supported by the demonstration that in the rat ECF-A is localised in the mast cells, probably in association with the granules (113). Human lung minced and extracted with butanol/glacial acetic acid solubilized large amounts of chemotactic activity which resolved into three peaks of chemotactic activity after Sephadex G-25 chromatography (MW > 10,000, 2000–4000 and 300–1000 Da). The latter peak had the same physico-chemical characteristics as ECF-A, and further purification steps resulted in two acidic tetrapeptides being identi-

fied as ECF-A (Val-Gly-Ser-Glu and Ala-Gly-Ser-Glu) (47). In later studies involving extraction with 1M NaCl and purification using high pressure liquid chromatography (HPLC) several other peptides were detected, suggesting that ECF-A is a family of closely related peptides especially as the two fully characterized peptides have rather weak and inconsistent activity (48). The eosinophilotactic activity of histamine has been the source of some controversy. Initial reports suggested it was inactive (64, 88). However, Clark et al. (19) reported that histamine was active over a dose range 10^{-7} M to 10^{-6} M. Turnbull et al. (105) also found that histamine had some activity although this was not very considerable and only observed at high concentrations (10^{-5} M). When these agents were applied to abraded skin the eosinophilic response was meagre when compared to specific allergen (11). LTB_4 is generally regarded as being the most active chemically characterized mediator of eosinophil locomotion (82, 116) and a recent report has suggested that it is released by antigen-challenged human lung (92).

We have examined the effect of PAF-acether on eosinophil locomotion using a modified Boyden chamber technique (62). PAF-acether was a very effective and consistent promoter of eosinophil locomotion over a dose range of 10^{-5} M to 10^{-8} M (optimum 10^{-6}), whereas lyso-PAF had minimal effect over the same concentration range. When compared with LTB_4 , histamine and the two ECF-A tetrapeptides, PAF-acether was considerably more effective in causing eosinophil locomotion (Fig. 2). Similar results were obtained irrespective of whether mixed populations of eosinophils or homogeneous populations of normal and light density eosinophils separated on metrizamide gradient were used as indicator cells. PAF-acether had both chemotactic and chemokinetic activity and produced high dose inhibition of eosinophil locomotion above 10^{-5} M (112). We have also investigated the effects of PGD_2 , a major mast cell-derived mediator, but this had little if any activity for eosinophils (Wardlaw, A., unpublished). PAF-acether was also able to enhance eosinophil-mediated cytotoxicity (schistosomula of *S. mansoni*) (75) as well as enhancing the expression of IgE and IgG

receptors measured using the rosette technique (110a). Intradermal injection of up to 800 pmol of PAF-acether into the skin of non-atopic normal volunteers resulted in an immediate wheal and flare reaction starting after 5 min and persisting for about 30 min. In 50% of subjects a delayed onset erythematous reaction was observed. Histological examination of the injection site after 4 h revealed an intense inflammatory infiltrate characterized by vasculitic lesions and infiltration with primarily neutrophils and some eosinophils and mononuclear cells (5). In contrast, injection of PAF-acether into the skin of atopic individuals resulted in the migration of large numbers of eosinophils into a skin blister chamber similar to the number observed with antigen (55). Furthermore, inhalation of PAF-acether by baboons was associated with the appearance of increased numbers of eosinophils in lavage fluid obtained by bronchoalveolar lavage 1 h after challenge with PAF-acether (26). Although PAF-acether in human systems does not appear to be primarily mast cell-derived there is one report of PAF-acether being released following challenge of dispersed human lung cells with anti-IgE (94). It is possible therefore that PAF-acether is partly responsible for the low molecular weight eosinophil chemotactic activity in supernatants derived from chopped lung challenged with allergen, described above as ECF-A.

The cell source of the eosinophil chemotactic activity which results in the infiltration of eosinophils into the airways after antigen challenge or in day-to-day asthma is also unknown. Allergen challenge to the skin which results in a late reaction is characterized by an influx of neutrophils followed shortly by eosinophils and then mononuclear cells after about 24 h (100). A similar pattern, at least with regards to eosinophils, also occurs in the upper and lower airways in association with a late response induced by inhalation of antigen (25, 30). Traditionally, it has been thought that this was due to mast cell-derived mediators because it was IgE-mediated and 48/80 could provoke a similar although considerably milder reaction (100). However, awareness that other cells also have functional IgE receptors (i.e. platelets, monocytes, lymphocytes and eosinophils) (17, 60, 78) and that potent mediators such

as PAF-acether and LTB_4 are primarily non-mast cell-derived must call this view into question. In addition, corticosteroids, which prevent the late reaction, do not prevent degranulation of lung mast cells *in vitro* (95) and beta-2 agonists such as salbutamol, which do partially prevent mast cell degranulation of both airway and parenchymal human lung mast cells (57, 111) *in vitro* and prevent the early response to antigen challenge, do not inhibit the development of the late response (20).

Mononuclear cells and eosinophil activation

It is becoming increasingly clear that monocytes and T lymphocytes have a crucial role in eosinophil maturation, activation and locomotion. T lymphocytes appear to be necessary for the eosinophilic response to parasite infection in animal models since eosinophil proliferation is abolished by thymectomy, thoracic duct drainage and anti-lymphocyte serum (8). In addition, in congenitally athymic mice infected with appropriate parasites an eosinophilia is not observed (21). Sensitized lymphocytes, contained within a chamber separated from the peritoneal cavity of mice by a Millipore filter, were able to induce an eosinophilia, suggesting that the effect was mediated by a soluble factor (8). Factors which caused migration of eosinophils *in vitro* were generated by mouse T lymphocytes after stimulation with both antigens and mitogen (22) and a similar factor has been described in humans associated with parasite infection (69). A factor derived from human placental conditioned medium was shown to stimulate eosinophil, neutrophil and monocyte colony formation (85). This factor termed CSF_α which has been cloned and has a molecular weight of 30 kDa also activates eosinophils, increasing their cytotoxic and phagocytic ability (28). CSF_α is also generated by human blood monocytes (107). Another mononuclear cell product (EAF) derived from unstimulated monocytes from subjects with moderate eosinophilia also enhanced eosinophil cytotoxicity and LTC_4 release (36, 108). This factor is an acidic protein with an approximate molecular weight of 40 kDa and is chemically similar to another factor derived from unstimulated human mononuclear cells by Dessein et al. (27). Recently, a mouse-

derived lymphokine with a molecular weight of 46 kDa has been described which is specific for eosinophil differentiation (93). This factor is effective at promoting human eosinophil differentiation and effector function, and it is likely that an analogous human cytokine exists. Several studies have described human mononuclear-derived factors which are chemotactic for eosinophils usually in the context of parasitic infection (69, 109). One interesting study described a factor derived from unstimulated T lymphocytes (with a molecular weight of 30–60 kDa) from the sputum of five asthmatics (89). Another group have found that T helper lymphocytes from the granuloma tissue and peripheral blood of patients with subcutaneous angioblastic lymphoid hyperplasia (SALH) spontaneously generate a factor which is chemotactic for eosinophils with a molecular weight of 40–60 kDa. T-lymphocytes from normal subjects also released a factor with a similar molecular weight after stimulation with concanavalin A (52). The precise role of these factors in asthma and other eosinophil-related diseases awaits clarification.

CLINICAL STUDIES

Bronchoalveolar lavage

In experienced hands bronchoalveolar lavage is a well tolerated and valuable tool for the investigation of basic mechanisms in asthma (84). Bronchoalveolar lavage has been performed both on asthmatics with day-to-day asthma and after inhalation challenge particularly with allergen. The response of sensitized asthmatics to challenge with inhaled allergen usually develops into one of two patterns. A proportion have a single early response of short duration, whereas about 40% have both a single and a delayed or late response which starts at about 3–6 h and is sustained for several hours. One of the first groups to perform bronchoalveolar lavage following antigen challenge found that late response was associated with a migration of eosinophils into the airways (25). No increase in eosinophils was seen in those subjects who had no reaction, those who only had an early response, or those who had

both an early and a late response who were lavaged at 2 h (instead of 6 h) before the onset of the late response. This suggests that eosinophils are associated with, and may be responsible for, the development of the late response. This observation has been confirmed by another group who also found an increase in the number of neutrophils in the lavage fluid (80). Animal models of the late response in the rabbit have also implicated granulocyte infiltration in the aetiology of the late response as well as induction of bronchial hyperreactivity, although the neutrophil appears to be more important in this system (81). Several investigators have reported changes in T cell subsets in association with allergen challenge in both blood and lavage fluid (91). One group found that there was an immediate fall in T-helper cells in the peripheral blood after antigen challenge which persisted for 72 h and a rise in activated (Ia positive) T cells after 48 h (40). These changes were not related to the pattern of the clinical response (i.e. single early reactions or dual response). Gonzalez et al. (30, 49) undertook similar studies performing allergen inhalational challenge on six asthmatics who had previously been documented as having single early reactions (SER) and six subjects who gave dual (early- and late-phase (LPR)) responses. Each asthmatic was lavaged twice. On the first occasion they inhaled the diluent (Dil) solution and after an interval of at least 7 days allergen challenge was undertaken. Lavage was performed on each occasion 6 h after challenge. Each subject therefore acted as their own control. There was a significant increase in the number and percentage of eosinophils, neutrophils and lymphocytes in the lavage fluid in the LPR ($P < 0.05$) after antigen challenge which was not observed in the SER. There was also a fall in the percent of mast cells in the LPR ($P < 0.05$). Intriguingly they observed a significant ($P < 0.05$) increase in the percentage and absolute number of OKT8 positive T cells in the lavage fluid from the single responders together with a decrease in the percentage of OKT4 positive T cells and the OKT4/T8 ratio ($P < 0.05$). In the peripheral blood there was an increase in the percentage of OKT4 T cells in the single responders. There were no differences between the diluent and anti-

gen challenge day in lymphocyte subsets in the dual responders in either the peripheral blood or the lavage fluid. These findings raise the possibility that mobilization of T suppressor cells into the lung after an allergen-induced single early response is associated with the prevention of a subsequent late-phase response into the airways after antigen challenge in those patients who have a single early response. An interesting hypothesis is suggested where antigen-stimulated T helper cell/macrophage interactions result in release of eosinophil activating factors which attract eosinophils into the airways where they degranulate and cause an inflammatory reaction expressed as the late response. In single early responders this reaction is then blocked by T suppressor cell-derived factors.

Studies of lavage in day-to-day asthma have also revealed a preferential increase in eosinophils in asthmatics with no consistent evidence of increased neutrophil infiltration (37, 46, 104). We have performed a cross-sectional study of mild atopic asthmatics to examine the cellular and mediator profiles in lavage fluid obtained during fiberoptic bronchoscopy (23, 67). One group of asthmatics were symptomatic at the time of lavage, requiring daily inhaled β_2 agonists. Although their lung function was well maintained they all had markedly hyperreactive airways (PC_{20} methacholine < 1 mg/ml, normal > 4 mg/ml). The second group had episodic asthma and were asymptomatic at the time of lavage. This group consisted of normoreactive and hyperreactive subjects (PC_{20} methacholine varying between 1 and 32 mg/ml). The two control groups were subjects with hay fever but no history of wheeze, and non-atopic normal individuals. There were six subjects in the hay fever group and eight in each of the other groups, and all the subjects were non-smokers. The symptomatic group had a significant increase ($P < 0.05$) in the percentage of eosinophils and epithelial cells in the differential count from both bronchoalveolar wash (obtained by inserting 180–240 ml normal saline into the lower lobe) and a bronchial wash (obtained by inserting 20 ml aliquot into the middle lobe). The epithelial cells were predominantly columnar, both ciliated and non-ciliated, although some squamous epithelial

cells were also seen. These differences were even more marked when the asthmatics who had hyper-reactive airways were compared with asthmatics and controls who had normoreactive airways ($P < 0.01$). We also measured the amounts of MBP in the lavage fluid of 15 of the subjects and found that these correlated with the percentage of eosinophils and were significantly raised in the hyperreactive asthmatics ($P < 0.01$). There were no differences in the percentage of neutrophils or lymphocytes, although there was a significant increase ($P < 0.01$) in the percentage of neutrophils in the bronchial wash compared to the bronchoalveolar wash in both the asthmatic and non-asthmatic groups. The difference, which has been noted by previous investigators, was in some cases very striking with cell populations reaching as high as 50% neutrophils. The increase in the percentage of epithelial cells is in agreement with the work of Laitinen et al. who found evidence of considerable epithelial damage in bronchial biopsies obtained from asthmatics undergoing rigid bronchoscopy (71). Epithelial damage is also a cardinal feature of biopsies from patients who had died from asthma (31). They may also be observed in the form of Creola bodies in the sputum of asthmatics which consist of clumps of epithelial cells (83). The epithelium forms both a mechanical barrier and may secrete a relaxant factor in a similar way to vascular endothelium (51). It is also a source of mediators (59). Stripping off the airway epithelium *in vitro* leads to an enhanced response to a variety of spasmogens (6). Loss of epithelial integrity, possibly mediated by MBP could therefore lead to bronchial hyperreactivity *in vivo*. However, we observed no correlation between the degree of hyperreactivity and the bronchoalveolar eosinophilia, suggesting that eosinophil-induced inflammation may be necessary for the development of hyperreactivity but is not the only factor involved.

Finally, Diaz et al., in a study of the effects of disodium cromoglycate (DSCG) on the constituents of bronchoalveolar lavage fluid in day-to-day asthma, found that the group given DSCG for 1 month had a significant decrease in the mucus eosinophil count which was not observed in the placebo group. Moreover when the responders to DSCG (on the basis of symptom scores) were compared with non-responders this change was

even more marked and observed in both the mucus and the bronchoalveolar eosinophils (29).

Peripheral blood eosinophilia

Durham & Kay (32) found that the degree of bronchial hyperreactivity inversely correlated with the peripheral blood eosinophil count in dual responders following antigen challenge, and these observations have recently been supported by a cross-sectional study of asthmatics attending a routine chest clinic where a similar correlation was observed (103). The presence of raised numbers of peripheral blood eosinophils in both atopic and non-atopic chronic asthma is well established although this elevation is not as great as in some other eosinophil associated diseases (9). One study confirmed that blood eosinophil counts in subjects with chronic asthma were elevated but counts were normal in a group of asymptomatic asthmatics (74). They also found that patients with acute severe asthma of sudden onset had an eosinopaenia which would be consistent with a large influx of eosinophils into the airways. Horn et al. in a longitudinal study of 14 oral steroid-dependent asthmatics attending the chest clinic on 44 separate occasions found that eosinophil counts correlated with several measurements of airflow obstruction (58).

Major basic protein

Sputum eosinophilia in asthma is also well recognized and this observation was extended by Gleich et al. who found that raised levels of sputum MBP were almost specific for asthma when routine sputum specimens were examined from 100 patients (38). Concentrations of MBP in some asthmatic sputa were of the same magnitude (10 g/l) as the concentrations which had caused epithelial damage *in vitro*. This group also found large amounts of extracellular MBP in biopsies of patients who had died of asthma often in presence of few intact eosinophils, suggesting that they had disintegrated at the site of the inflammatory process (34). Extracellular MBP was not seen in those patients with asthma who had no symptoms at the time of death and non-asthmatic controls. These studies suggest that

markers of eosinophil products may in some circumstances be a more appropriate way of looking for evidence of eosinophil involvement than intact eosinophils.

Corticosteroids

The action of steroids also provides indirect evidence of a causative role for eosinophils in asthma. Corticosteroids are the most effective drugs for the treatment of chronic asthma. They also cause an eosinopaenia in normal subjects and asthmatics (58). The mechanism for this is unclear. In one study intravenous injection of 100 mg of hydrocortisone caused a profound eosinopaenia within 4 h which persisted for 24 h (50). Such a rapid fall suggests that margination within the vascular epithelium may be involved; however, corticosteroids are effective in suppressing production of lymphokines and mono-

kines which may be involved in eosinophil activation in asthma (99). Another recent study has found that corticosteroids also suppress eosinophil colony formation from peripheral blood and bone marrow but promoted neutrophil colony formation (10).

Aspirin sensitivity

The association of aspirin sensitivity, asthma and marked peripheral blood eosinophilia is well recognized (41), and bronchoalveolar lavage in these patients suggests that they have a considerable airway eosinophilia (46). As with other drug reactions associated with an eosinophilia the mechanism is unknown although it does not appear to be IgE-mediated. I have not mentioned in this review asthma syndromes associated with other pathological events, such as allergic bronchopulmonary aspergillosis or Churg-Strauss.

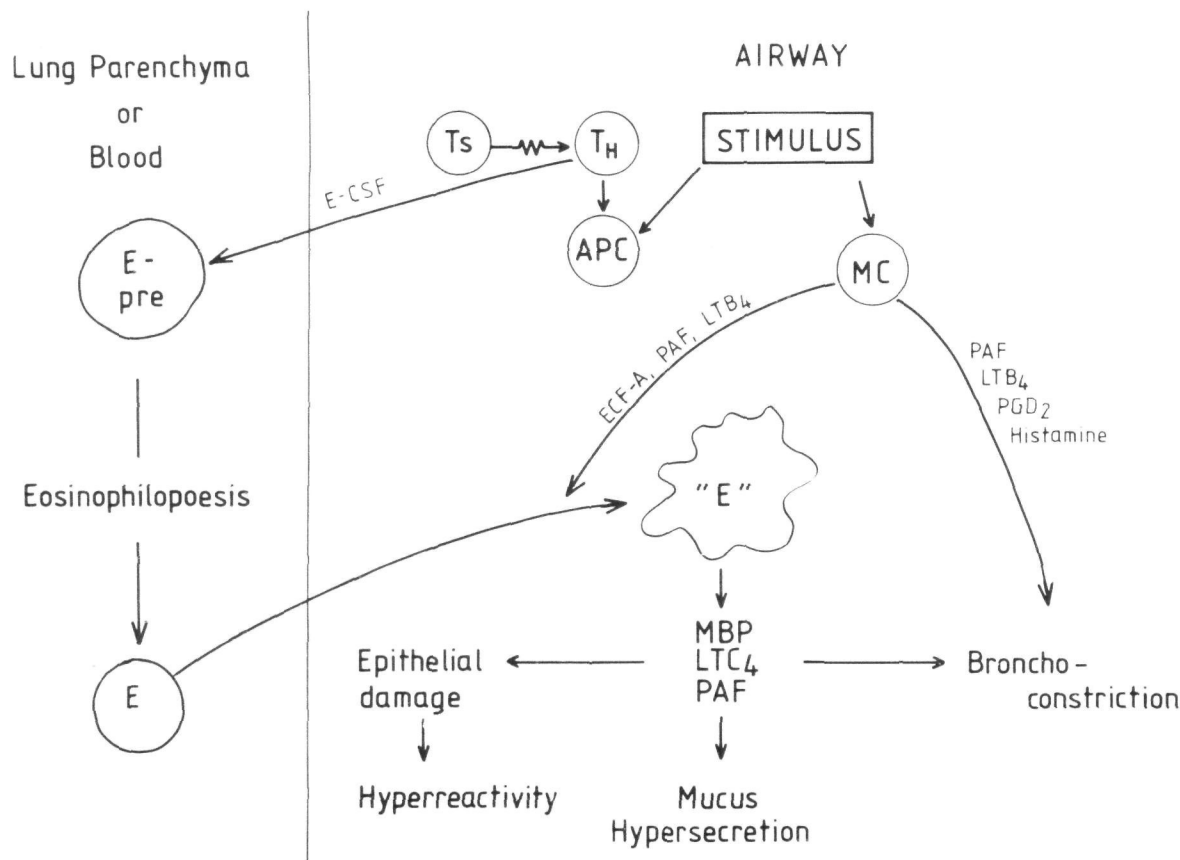


Fig. 3. Scheme to illustrate a possible mechanism of eosinophil-induced inflammation in asthma. A stimulus (e.g. specific allergen) interacts with a mediator cell (MC) (e.g. mast cell or alveolar macrophage). Mediators released cause bronchoconstriction and eosinophil accumulation and activation in the airways. Eosinophil accumulation is augmented by local eosinophilopoiesis under the influence of T helper cell-derived (T_H) colony stimulating factors (E-CSF) generated following interaction with antigen presenting cells (APC). This pathway may be suppressed by T suppressor cells (T_s) as well as corticosteroids. E-pre: Eosinophil precursor; E: Eosinophils; "E" activated eosinophil.

These are even more strongly associated with a tissue and blood eosinophilia than uncomplicated asthma. Relatively few studies have specifically examined the role of the eosinophil in these conditions, although it is likely that the observations made above are of relevance to their pathogenesis.

CONCLUSION

The circumstantial evidence implicating the eosinophil as the major effector cell in asthma is considerable. Eosinophils are clearly associated with day-to-day asthma both in its mild and severe manifestations. They are also associated with models of asthma, particularly the late response to antigen challenge, and bronchial hyperreactivity which is a hallmark of asthma. Eosinophils can generate mediators whose actions might evoke many of the pathological features of asthma, such as epithelial damage, mucus production, oedema and bronchospasm, and their cytotoxic capacity is clearly revealed in their capacity to kill parasites *in vivo* and *in vitro*. Indeed several investigators have suggested that their teleological role is to protect against parasites and that in asthma they have become inappropriately triggered (65). A decrease in eosinophil counts in the blood and airways is associated with response to treatment with corticosteroids and DSCG. Several inflammatory mediators have been shown to activate eosinophils, particularly PAF-acether, LTB₄ and mononuclear cell products, although *in vivo* studies are few. Attraction of eosinophils into the airways in asthma may be a two-stage process initially involving increased eosinophilopoiesis in the bone marrow, blood or lung parenchyma and then more locally active mediators attracting the eosinophil to the site of the inflammatory response (Fig. 3).

Eosinophils appear to be equally important in atopic and non-atopic asthmatics, which supports the view that tissue damage in extrinsic and intrinsic asthma is mediated by a final common pathway. Several other lung diseases such as fibrosing alveolitis and pulmonary eosinophilia are associated with a similar or even greater elevation in lung eosinophil counts without evidence of bronchial asthma. This may reflect the possibility that different triggers produce dif-

ferent responses from the eosinophil. However, it is more likely to be due to the different site of activation, bronchial versus interstitial, the cause of which remains unknown and is one of the many enigmas which obscure our understanding of the pathogenesis of this fascinating disease.

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