

The Role of the Eosinophil Granulocyte in the Inflammatory Reaction

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Exactly one hundred years ago, Paul Ehrlich (27) characterized the eosinophil granulocyte by its peculiar affinity for the acid dye eosin. Despite numerous investigations (Schwartz' review of 1914 contained 2,758 references) (62), it is still a mysterious cell. It seems reasonable to expect some analogy with the neutrophil granulocyte as both cells are constructed in a similar fashion. The neutrophil granule content is thought to act primarily on micro-organisms that have been sequestered in phagocytic vacuoles. By analogy the eosinophil may have a primary role in killing of parasites. Evidence also indicates extracellular release of granule constituents from neutrophils; immune complexes on a surface and even soluble factors (e.g. C5a) can be signals for degranulation. It is possible that the eosinophil is designed for extracellular secretion as it is not a very good phagocyte. Signals for degranulation may be similar for both neutrophils and eosinophils. However, the specific eosinophil content may act primarily on extracellular substrates for useful modulation of immunological reactions and destruction of parasites. On the other hand, it has become increasingly clear that, like the neutrophil, the eosinophil can sometimes give rise to non-useful tissue damage and degradation.

The purpose of this review is to focus on the biochemical and biological properties of eosinophil granule constituents because some new knowledge has recently developed in this

field. We will discuss the evidence for the participation of the eosinophil in the allergic response and other inflammatory reactions like that of parasite infections. The mechanisms of eosinophilia will be commented on.

EOSINOPHIL PRODUCTION

Granulopoietic stem cells can be demonstrated by formation of colonies *in vitro* by culture in agar in the presence of colony-stimulating factors (CSF). CSF are heterogeneous and can be produced by a variety of cells like macrophages, endothelial cells and T-lymphocytes (51). A heterogeneity also exists among colony-forming cells, thus neutrophil and eosinophil colonies arise from separate colony-forming cells. This is in agreement with the occurrence of normal or even increased eosinophil production in congenital genetic agranulocytosis (44). A considerable number of eosinophil colony-forming cells is normally present (37). Therefore, it remains to be explained why eosinophil production is lower than neutrophil production. The answer may be a lower normal proliferation rate of eosinophil than neutrophil colony-forming cells. The stimulators of eosinophil production appear to be distinct from those for neutrophils. Antigen or nitrogen-stimulated lymphocytes could be the primary source of CSF stimulating eosinophil colony-forming cells. Eosinophil production *in vivo* is increased by production of a factor released by activated T-

lymphocytes (14). This factor may be identical with specific eosinophil stimulating CSF.

Depletion of mature eosinophils by administration of anti-eosinophil serum generates a low molecular weight peptide, which stimulates eosinophilopoiesis *in vivo* (48). It was suggested that this substance be named eosinophilopoietin.

Eosinophil transit time in the blood is only a few hours for most species (22). Blood to tissue ratios of eosinophils has been estimated to be 1:100 in man (54).

GRANULE FORMATION

During maturation in the bone-marrow, eosinophils synthesize a number of proteins that are transported to the Golgi apparatus and packaged in granules destined for secretion into phagocytic vacuoles or extracellular space. The eosinophil, like the neutrophil, produces two types of granules in separate stages of differentiation—one during the promyelocyte and another during the myelocyte stage (10). Large spherical homogeneous primary granules are formed in the eosinophil promyelocyte. The specific granules with the peculiar affinity for eosin are formed in the myelocyte. This second granule population consists of typical irregular, crystalloid-containing granules, which are peroxidase positive. Specific granules are predominant in mature eosinophils.

GRANULE COMPOSITION

The content of eosinophil granules has been evaluated both by cytochemical techniques (10) and subcellular fractionation in combination with biochemical techniques (4, 53, 79). Neutrophil and eosinophil granules differ considerably in composition. Eosinophils lack lysozyme and lactoferrin, which are major constituents of neutrophil specific granules. They also lack the group of neutral proteases, which are a major part of the azurophil granules of neutrophils (3). A variety of hydrolytic enzymes like beta-glucuronidase,

acid phosphatase, DNA-ase, RNA-ase and other lysosomal enzymes are present in both eosinophil and neutrophil granules (4).

Peroxidase is especially abundant in eosinophils (3). The finding of a different absorption spectrum of the reduced form of eosinophil peroxidase as compared to neutrophil myeloperoxidase suggested the existence of two distinct enzymes. This is supported from studies of genetic deficiency of myeloperoxidase; peroxidase is absent from neutrophils and monocytes but present in eosinophils (61). The physiological substrate for eosinophil peroxidase must be regarded as unknown.

The human eosinophil has been held to be the major source in peripheral blood of *arylsulphatase* (68). The eosinophil enzyme (77) had the characteristics of a type IIB arylsulphatase; thus the apparent molecular weight was 60,000, it cleaved preferentially 4-nitrocatechol sulphate over *p*-acetyl-benzene-sulphonic acid, it was inhibited by phosphate ions and it had a pH optimum of 5.7.

Data on the type of *phospholipase* (55) in eosinophils are conflicting. A recent report (40) indicated the presence of phospholipase D, previously only detected in plants.

Plasminogen has been reported to be present in eosinophils (11).

Eosinophil cationic proteins

Early studies (76) suggested the presence of an arginine-rich substance in eosinophils. It was claimed to have a weak antihistamine effect. Further characterization was reported in the elegant work of Gleich et al. (29, 30), who isolated a major basic protein of eosinophil granules from guinea pigs. This accounted for greater than 50% of the total granule protein. The major basic protein readily aggregated and became insoluble, and the formation of aggregates depended on the establishment of disulphide bonds. It was suggested to constitute the large crystalline core of eosinophil granules

seen with electron microscopy. This suggestion has actually been confirmed (45). Analysis of reduced and alkylated major basic protein in 6 M guanidinium chloride revealed a single polypeptide chain with a molecular weight of 10,800. Amino acid analysis showed the presence of 13% arginine. Two free sulphydryl groups were found per molecule basic protein.

Despite the fact that the major basic protein is the principal constituent of guinea pig eosinophil granules, it has so far not been associated with any unique biological activity (29). It did not increase vascular permeability when injected in the skin, nor did it antagonize the effect of histamine and bradykinin in the skin. Furthermore, it did not contract the isolated guinea pig ileum and did not in the same test system inhibit the action of histamine or bradykinin. The marked basicity in combination with the presence of two available sulphydryl groups suggested that the protein might interact with acidic molecules and enzymes depending on sulphydryl groups for their activity. The major basic protein actually activated papain more efficiently than cysteine. By ionic interaction the anticoagulant activity of heparin was neutralized.

We have identified arginine-rich proteins also in human eosinophil granules (52, 53) designated eosinophil cationic proteins (ECP). The major component had a molecular weight of 21,000. The isoelectric point is above pH 11. These compounds show approximately 2.5 moles zinc per mole protein. Thus the high zinc content of eosinophils is likely to depend on the presence of the eosinophil cationic proteins. Approximately 30% of the protein content of the granules belong to this group of eosinophil constituents. No bactericidal or esterolytic activities were detected. Nor did ECP inhibit guinea pig ileum anaphylatoxin or histamine-induced contraction.

Human ECP seems to be analogous with the major basic protein of guinea pig eosinophils. The molecular weights are, however, different. Both contain similar amount of arginine consistent with the basic character of the molecules; cysteine content is also similar but

the aspartic acid content is much higher in human ECP whereas the content of glycine is lower. The presence of a major basic protein also in human eosinophils was recently reported by Gleich et al. (31). Its molecular weight was 9,200, it was also rich in arginine and readily formed disulphide-bonded aggregates. The relationship between this protein and ECP is not clear at present.

In vitro experiments showed that ECP could affect several humoral mediators of inflammation (Fig. 1). Minute amounts shortened the time for plasma coagulation (72). This effect is probably mediated by activation of coagulation factor XII (Hageman factor) or factor XII-related compounds. Furthermore, activation of pre-kallikrein in plasma as measured by the formation of kallikrein-activity, was greatly enhanced by ECP. That finding further supports the assumption that ECP is active against factor XII-related compounds.

Activation of plasminogen can be brought about by substances like urokinase. The rate of urokinase-mediated activation was considerably increased by pretreatment of plasminogen with ECP (25). ECP itself was not capable of activating plasminogen to plasmin. The effect of ECP demonstrated here is similar to that described for some synthetic substances (63) and for a tissue compound (8). The mechanism seems to be preactivation of plasminogen by transforming it from the Glu-form to the Lys-form (20, 80). Preactivation of plasminogen has been proposed to be of importance for increasing the efficiency of the fibrinolytic system *in vivo* (7, 71). The eosinophil may therefore be of importance in this context.

We have developed a radioimmunoassay for detection of human ECP in serum (73). The normal range is 5–55 $\mu\text{g/l}$. A correlation was found in healthy individuals between eosinophil counts and ECP of serum. ECP seemed to appear in serum both as free protein (major part) and in complex with an unknown serum constituent. However, both free and complexed protein showed identical immunoreactivity.

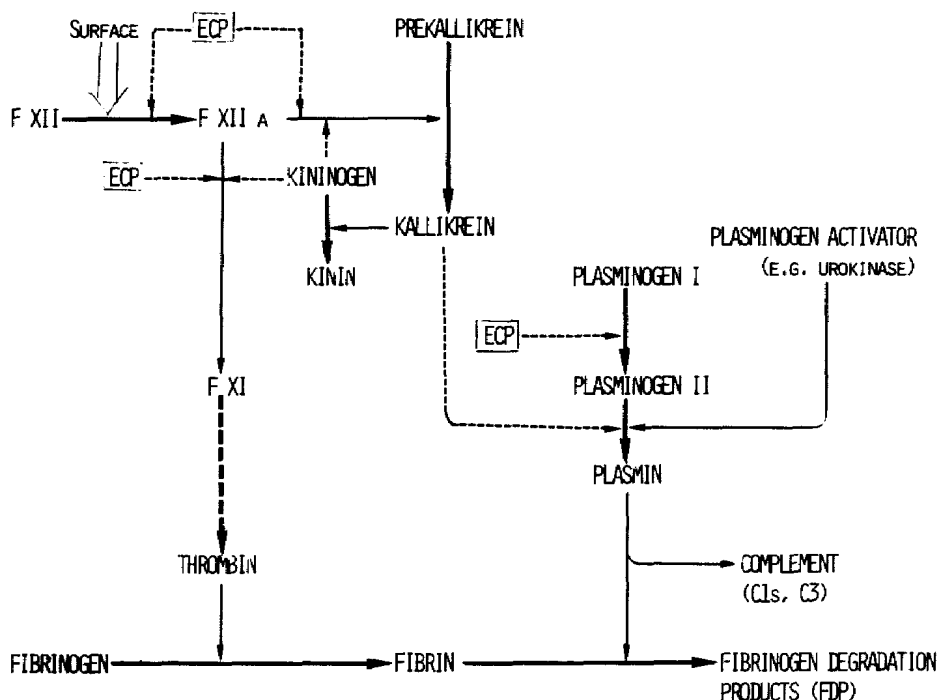


Fig. 1. Effects of "eosinophil cationic protein" (ECP) on coagulation and systems related to coagulation. Enzyme activity ($\rightarrow$). Co-factor activity by uncertain mechanism ($\dashrightarrow$). Proteolytic or conformational alteration of a protein molecule ($\Rightarrow$). Plasminogen I and II are tentatively the Glu and Lys-forms respectively.

EOSINOPHIL MIGRATION AND ACCUMULATION

The eosinophil is a motile cell, which accumulates within hours at the site of an inflammatory focus such as an allergic reaction, delayed hypersensitivity reaction, or acute infection (15). The stimuli leading to accumulation of eosinophils *in vivo* are not known. However, several factors have been demonstrated to attract eosinophils *in vitro*. Some of these attract neutrophils equally well like complement-derived components C3a, C5a and the trimolecular complex C567. Among factors that attract eosinophils preferentially are Eosinophil Chemotactic Factor of Anaphylaxis (ECF-A) (42) and Eosinophil Stimulation Promotor (ESP) (24). ECF-A is a preformed tetrapeptide secreted by IgE-

sensitized mast cells or basophils after exposure to a specific allergen (41). It is therefore one possible candidate to make responsible for the accumulation of eosinophils at the site of an allergic reaction. ESP is a lymphokine secreted by sensitized T-lymphocytes (38) and might be responsible for migration of eosinophils to sites of delayed hypersensitivity.

Histamine has long been regarded as a potent eosinotactic substance (15). However, most of the experiments with histamine have been made utilizing cells from non-human species (6). The eosinotactic activity is not established for human cells (15). But it is of considerable interest that histamine may increase the responsiveness of human eosinophils to active complement components (21).

Eosinotactic activity has been demonstrated also in cell cultures and extracts from certain

malignant tumours accompanied by eosinophilia (15).

PHAGOCYTOSIS AND EXOCYTOSIS

Eosinophils can phagocytose a wide variety of substances (15, 22). As with neutrophils, ingestion is followed by degranulation with release of granule contents into the phagocyte vacuole. However, phagocytosis with eosinophils is obviously much less efficient than that of neutrophils (15). One could wonder if this cell is designed for phagocytosis and intracellular killing. Eosinophils give inferior protection against bacterial infection even when increased in numbers as they often are in congenital genetic agranulocytosis. Eosinophils are said to show efficient ingestion of immune complexes or mast cell granules. The ingestion of these materials is, however, not unique for eosinophils. Therefore, it is attractive to suggest primarily an extracellular function for eosinophil granule constituents. Such a function is satisfied better by a cell with well-developed capacity for exocytosis as compared to phagocytosis and could be useful both for destruction of parasites and modulation of inflammatory reactions.

Even if eosinophils are not very good phagocytes the oxidative response to phagocytosis of bacteria is greater in the eosinophil than in the neutrophil (9). This corresponds to a high production of hydrogen peroxide, which could have a cytotoxic effect on parasites and a modulatory effect on mediators of inflammation. The very high level of resting eosinophil iodination may be related to the high cellular peroxidase and the high resting metabolism with abundant peroxide production (16).

If the eosinophil should be viewed as a secretory cell we need to know more about regulation of secretion by this cell. Actually, ingestion of complexes formed of human immunoglobulins with specific rabbit antibodies showed release of less than 5% of the peroxidase content (69). Complexes formed by IgE were a little more effective than those

formed with IgG and IgM. Ingestion of complexes of IgE might lead to suppression of the allergic reaction by means of extracellular deposition of eosinophil peroxidase. Eosinophil degranulation has also been noted into the extracellular medium on confrontation with antigen-antibody complexes too large to ingest (5). Arylsulphatase was found to be released upon phagocytosis of starch particles coated with serum (77). ECF-A itself initiated release of substantial amounts of arylsulphatase (77). The latter finding points to the possibility that eosinophil secretion is primarily regulated by humoral factors.

Receptors for complement are found on eosinophils from the blood of both normal individuals and patients with eosinophilia (34, 56, 67). Eosinophils, however, do not have strong immune adherence receptors for C3b and C4b (unlike neutrophils) but do have C3d receptors. It also seems that normal eosinophils have a different IgG binding capacity than neutrophils as the former cells do not form rosettes with rabbit IgG-coated erythrocytes (67). Eosinophil Fc receptors are of such low apparent affinity that they can only bind aggregated IgG (34). However, unlike normal eosinophils, eosinophils in the blood of patients with an eosinophilia have receptors that bind to the Fc region of human IgG1 and IgG3 (67). It was suggested to depend on "activation" or maturation of the eosinophil and was paralleled with signs of degranulation, vacuole formation and increased nuclear segmentation.

FUNCTION OF THE EOSINOPHIL IN IMMEDIATE HYPERSENSITIVITY

Local accumulation of eosinophils and peripheral blood eosinophilia are typical signs of an allergic reaction. Fig. 2 depicts the interaction between mast cells or basophils and eosinophils suggested to be of importance in the IgE-mediated immediate hypersensitivity reaction. The eosinophil may have regulatory functions that could modulate or eventually terminate the allergic reaction. Indeed several

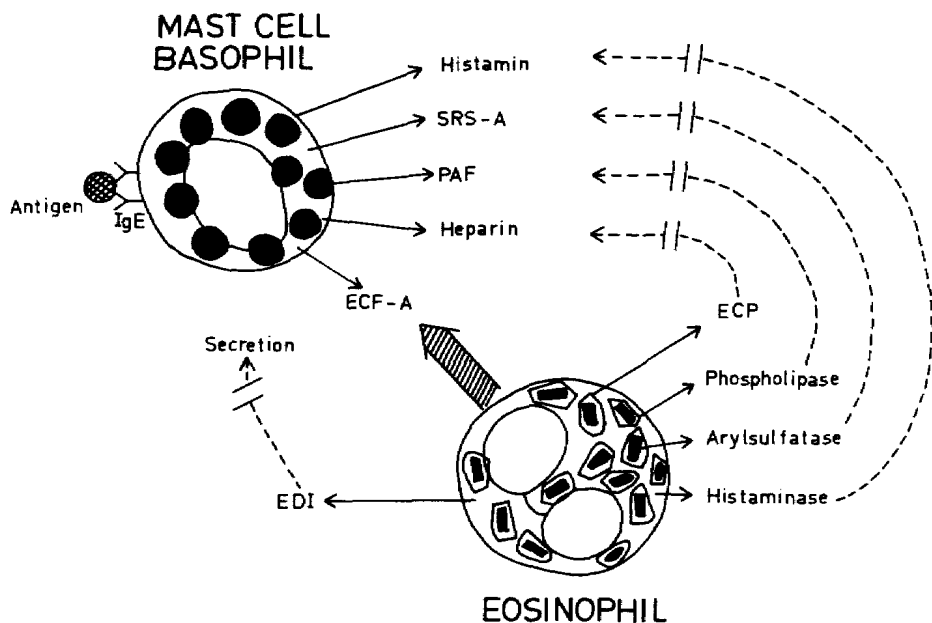


Fig. 2. Possible modulation of the immediate hypersensitivity reaction by eosinophil products. ECF-A, eosinophil chemotactic factor of anaphylaxis; SRS, slow reacting substance of anaphylaxis; PAF, platelet activating factor; ECP, eosinophil cationic protein; EDI, eosinophil derived inhibitor.

constituents of eosinophil granules can neutralize specific products of mast cells released when antigens react with cell-bound IgE. The mast cell products include histamine, "slow reacting substance of anaphylaxis" (SRS-A), ECF-A, "platelet activating factor" (PAF) and heparin (33). The extracellular deposition of products from the eosinophil can occur as a result of ingestion of antigen-antibody (IgE) complexes or mast cell granules. However, secretion of eosinophil products as mediated by humoral factors originating from mast cells and basophils may also be important (77).

Once in the focus of the allergic reaction the first action of the "activated" eosinophil could be to release substances that inhibit further basophil degranulation. An "eosinophil-derived inhibitor" (EDI) has been shown to inhibit histamine release by human leukocytes exposed to anti-IgE or specific antigen (36). EDI seemed to be preformed in the eosinophil. The effect on histamine release was paralleled

by activation of adenylate cyclase, thereby increasing cAMP, which blocked further mediator release. EDI appears to be mixture of E_1 and E_2 prostaglandins. However, this is only one of the possible actions of the prostaglandins. They could have many more effects in the allergic reaction, not only of an inhibitory character.

Eosinophil *arylsulphatase* was claimed to provide a major source of the enzyme responsible for inactivation of SRS-A at the site of an allergic reaction (77), SRS-A having a free sulphate group. The enzymatic nature of the interaction between eosinophil arylsulphatase and SRS-A was indicated by the pH and time-dependent linear inactivation.

The platelet activating factor (PAF) of mast cells and basophils was characterized as a phospholipid of low molecular weight (39). It causes the secretion of amines from rabbit platelets. The *phospholipase D* of eosinophils was able to cause a time-related inactivation of

PAF (40). It is not clear, however, if significant effect can occur *in vivo*.

Several observations *in vivo* have indicated that eosinophils have an *antihistamine* effect (15). But it is uncertain whether these effects are direct or indirect. Otherwise histaminase activity has been demonstrated both in eosinophils and neutrophils (81). There are also non-eosinophil sources of arylsulphatase.

Eosinophil cationic proteins (ECP) are specific products of eosinophils and may have some effects on the allergic reaction by neutralization of heparin.

We do not know whether the ability of eosinophil constituents to antagonize mediators of inflammation *in vitro* is relevant to their function *in vivo*. The eosinophil may be called on primarily to take responsibility in elimination of the allergen.

Effects of eosinophils on coagulation

Fibrin deposition is a very typical feature of inflammation. Two cells are almost invariably found in close connection with deposited fibrin, namely neutrophils and eosinophils (58). The eosinophil has been suggested to participate in fibrinolysis (60). This role of the eosinophil has been further substantiated by the finding of ECP-mediated preactivation of plasminogen as described above. Seemingly contradictory to this interpretation is the suggestion of procoagulant activity found in eosinophils (46) and the suggestion of a direct role for the eosinophil in the fibrin deposition in allergic reactions (59). Obviously much work remains to clarify the role of the eosinophil in coagulation.

SERUM LEVELS OF EOSINOPHIL CATIONIC PROTEINS IN ALLERGIC REACTIONS

Assays of serum-ECP have given some information, which may reflect the role of the eosinophil in allergic reactions. Fig. 3 shows variations of eosinophil blood counts and serum-ECP upon inhalation challenge test on a patient with allergic asthma (26). Concomitant with the initial symptoms there is

a decrease in eosinophil counts which may depend on eosinophil margination or migration into the tissues. Surprisingly, a concurrent great increase is seen for serum-ECP, with a peak 30–60 min after the challenge. The increase of serum-ECP could be due to release from circulating eosinophils or from eosinophils activated locally in the lung. In the former case, substances produced in the allergic process and released to the circulation could mediate secretion by the eosinophils. After peak level, serum-ECP decreases very rapidly indicating active elimination from the circulation. During the late allergic reaction (demonstrated in Fig. 3 by decreasing PEFR) there is a large increase in eosinophil counts. Oddly enough, an increase in serum-ECP is not found; in health there is a close relationship between eosinophil counts and serum levels (73). Several possible explanations for the finding in asthma come to mind; increased rate of elimination or inhibition of protein release from circulating and tissue eosinophils are the most likely. An analogous situation was found in patients with clinical asthma (75). Especially in patients with a combination of intrinsic asthma and hypersensitivity to aspirin, eosinophil protein of serum was much lower than expected. Indeed there was a suggestion of an inverse relation between eosinophil protein of serum and severity of symptoms.

Studies of serum-ECP may have some importance for research on the allergic process. However, the interpretation of our findings is difficult, in particular because of lack of data on turnover rate of serum-ECP. If a typical pattern can be established in immediate type hypersensitivity, determinations of serum-ECP may become of importance in obscure hypersensitivity reactions.

FUNCTION OF EOSINOPHILS IN DIVERSE TYPES OF INFLAMMATORY REACTIONS

A rapid decrease in the number of circulating eosinophils is a characteristic sign of an acute

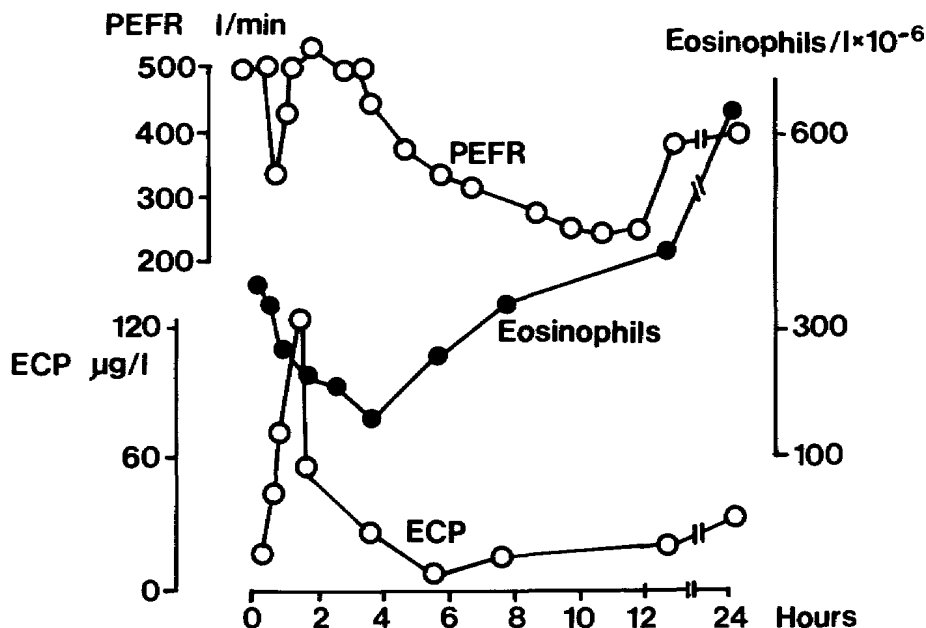


Fig. 3. Variations of eosinophil counts and serum-ECP during inhalation challenge with allergen in allergic asthma (45). PEFR, peak expiratory flow rate. Concomitant with initial symptoms (initial dip of PEFR) there is a decrease of eosinophils but a peak of serum-ECP. During the late allergic reaction (low PEFR) there is an increase of eosinophil counts without a concomitant increase of serum-ECP.

infection (15) or an inflammatory reaction of the type seen in acute myocardial infarction (43). This eosinopenia has been suggested to depend on concomitant rise of the cortisol level (70). Cortison administration has a well-known eosinopenic effect (78). In a series of elegant experiments Bass (12, 13, 15) showed that eosinopenia of acute infection was evoked also after adrenalectomy, which suggests that the eosinopenia is not explained by a rise of cortisol. The results suggested that it was caused by an attraction of the eosinophils to the site of infection. The eosinophils accumulated at some distance from the focus of the inflammation and seemed to surround the process. The cell may in some way be actively involved also in this kind of inflammatory process. Our findings of a high level of serum-ECP during acute infection in spite of the absence of circulating eosinophils (74) is consistent with an active involvement of the

eosinophil in the inflammation. The eosinophils could liberate their granule content at the site of the inflammation by means of active secretion or disintegration of the cell.

In patients with acute myocardial infarction we have also observed (35) remarkable peak-like rises of serum-ECP during the first 2-3 days of the disease in spite of the absence of blood eosinophils. These findings too suggest that the eosinophil is actively involved in inflammatory processes.

THE EOSINOPHIL AND PARASITE INFECTION

The finding that an anti-eosinophil serum abolished the immunity of mice infected with *Schistosomiasis* (49) pointed to a unique role of eosinophils in the defence against parasite infection. Antisera to lymphocytes, neutrophils or macrophages had no such effect. Human

eosinophils from normal individuals were reported to be the principal effector cell in antibody (IgG)—dependent *in vitro* killing of schistosomula (17) as measured by a ^{51}Cr -release assay. Subsequent work (2) showed that the eosinophil was not the only human blood cell that mediated antibody-dependent schistosomula damage. Preferential killing of schistosomula by the human eosinophil, as compared to the neutrophil was demonstrated only when complement was present either alone or in combination with antibody. With antibody alone the percentage killing was relatively small and equal by both types of granulocytes. It is important that schistosomula can be coated with C3 in the absence of antibody through activation, by the tegument, of the alternate pathway of complement after incubation of schistosomula with fresh rat serum (57). This was found to lead to a higher susceptibility to damage by rat eosinophils as compared to IgG-coated schistosomula.

The eosinophil-dependent cytotoxicity mechanisms seem to require a signal provided by soluble mast cell mediators (19). Soluble mediators released after mast cell activation by compound 48/80 or by IgE were as effective as intact mast cells. Therefore, mast cell mediators released *in vivo* could have an eosinophilotactic activity as well as an enhancing effect on the eosinophil cytotoxicity. In fact, it has been shown that the expression of complement receptors on human eosinophils is enhanced or unmasked by pharmacological mediators like ECF-A tetrapeptides and histamine (1). This finding led to the idea that a major biological function of these mediators is to render eosinophil complement receptors more available, thereby increasing the magnitude of adhesion of eosinophils to opsonized parasites (1).

After attachment to the parasites as mediated through complement receptors, the granule content is discharged on to its substrate at the surface of the parasite. One can only speculate on specific action *in vivo* of constituents of eosinophil granules like peroxidase, ECP and products of the reductive

oxygen metabolism. Major basic protein of eosinophils has some *in vitro* cytotoxic effect on *Schistosoma mansoni* (18). Although the finding that anti-eosinophil serum can abrogate resistance to a parasite infection (49) is very intriguing, the eosinophil does not seem to be the only cell active in the destruction of parasites.

There is an analogy between the reaction at the focus of a parasite infection and the immediate hypersensitivity reaction. In both instances the eosinophils may be recruited by products of the mast cell released by interaction between antigen and IgE on the surface of the latter cell. Mast cells have also been shown to adhere to complement-coated schistosomula (64) leading to release of factors which can mobilize and activate the eosinophil. A model for the interaction between parasite, mast cell and eosinophil is depicted in Fig. 4.

EOSINOPHILIA

Eosinophilia is most often observed in pathological states where there is an interaction between antigen, IgE antibody and mast cells or basophils. However, no causal relationship exists between levels of IgE and eosinophils. IgE myeloma does not give eosinophilia. The induction of the increased eosinophil production seems to be an immunological phenomenon involving thymus-processed lymphocytes (15). A memory for prior experience with antigens is indicated, as a greater production of eosinophils follows a second challenge with the same agent (15). This is not to say that eosinophils show immunological specificity in their function; it is only the stimulus to increased eosinophil production that is specific. A summary of the conditions in which eosinophilia occurs frequently is found in Table 1.

The association of eosinophilia and invasive metazoan infection is almost uniform. The eosinophils have been thought to modulate the intense local granulomatous reaction (15) or assist in the host's destruction of the parasite invaders as discussed above.

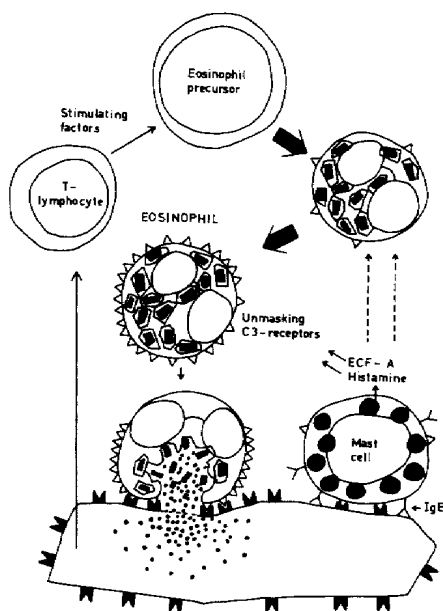


Fig. 4. Killing of parasite by eosinophil. The parasite is coated by C3 through activation of complement by the classical or alternate pathway. Mast cells can adhere to the parasite through C3-receptors. By interaction between antigen and IgE on the surface of mast cells, mediators like ECF-A are released leading to accumulation of eosinophils. C3-receptors on the eosinophils are unmasked by the action of ECF-A and histamine leading to more efficient attachment to the opsonized parasite. Eosinophil granule constituents are released on to the parasite and killing is achieved by unknown mechanisms. An eosinophilia is produced by activation of bone marrow eosinophil precursors through products of activated T-lymphocytes. This model is based on experimental studies reviewed in the text.

The eosinophil may also serve as an effector cell in the tissue damage found for example in tropical eosinophilia. In this disease the serum IgE level and the blood eosinophil count are usually very high. Microfilariae have been found in lung biopsies containing granuloma. The suggestion that the eosinophil participates in tissue damage is based on alterations in blood eosinophils, which make them activated, and altered distribution and kinetics of these cells (65). The mechanism for the destructive power is likely to be found in granule

constituents. Similar mechanisms may explain the tissue injury of eosinophil gastroenteritis.

The link between atopic states and eosinophilia is IgE, which provides the mast cell degranulation. Raised IgE is also often associated with allergy to drugs. Perhaps in most instances the only clinical evidence of drug allergy is the eosinophilia. However, several clinical signs may occur like fever, skin lesions, infiltrations of the lung, asthma, hepatic injury and renal injury (15). The pulmonary infiltration with eosinophilia (PIE) syndrome is mediated through IgE or immune complexes.

Peripheral blood eosinophilia is an uncommon occurrence in malignant tumours. However, some patients have been described with an anaplastic squamous cell carcinoma of the human lung associated with peripheral eosinophilia and eosinophilic infiltration of the tumour. A low molecular weight peptide chemotactic for eosinophils was isolated from the tumour cells (32). This peptide differed only slightly from the mast cell-associated tetrapeptides of the ECF-A and may represent ectopic production of a mediator of eosinophil chemotaxis. It is not clear whether the

Table 1
Conditions associated with eosinophilia

Parasite infections
Atopic disease (asthma, seasonal rhinitis, dermatitis)
Drug allergy
Urticaria
Pemphigus
PIE-syndrome:
a) Löfller's syndrome
b) Tropical eosinophilia
c) Pulmonary infiltrates with asthma
d) Chronic eosinophilia with pulmonary infiltrates without asthma
e) Vasculitis (e.g. polyarteritis nodosa)
Immune complex diseases
Eosinophil granuloma
Eosinophil gastroenteritis
Eosinophil fasciitis
Occasionally with carcinoma, sarcoma and Mb Hodgkin
The hypereosinophilic syndrome
Eosinophil leukemia

tumour-derived peptide itself or a separate tumour-derived eosinopoietic factor, or both acting in concert, induced stimulated production of eosinophils.

The hypereosinophilic syndrome

Persistent eosinophilia (of at least 1.5×10^8 eosinophils/l for longer than 6 months) is typical for this syndrome. In addition it is characterized by infiltration in a varying frequency of liver, spleen, heart, lung, kidney and skin. The heart is the organ most seriously affected with dense endocardial and myocardial fibrosis, Löffler's cardiomyopathy (47). Prolonged high eosinophilia from any cause can produce this unique type of cardiomyopathy. Adaptive selection during evolution has perhaps led to the normal production of a lower number of eosinophils than neutrophils to avoid tissue injury. Unlike eosinophils from normal individuals eosinophils from patients with Löffler's cardiomyopathy possessed receptors for Ig and C3b (66). Moreover the eosinophils were vacuolated and contained reduced numbers of crystalloid granules. Also ultrastructural changes have been reported with reversed stainability of granules, making the central crystal lighter than the surrounding matrix (50). It was suggested (66) that the restrictive cardiomyopathy of hypereosinophilic states is a result of prolonged release of products from degranulated eosinophils while they are in the circulation. This hypothesis remains to be tested.

The high eosinophilia syndrome could be regarded as a myeloproliferative disorder connected with the unusual eosinophilic leukemia. Perhaps manifest leukemia does not have time to develop in most cases because of fatal cardiomyopathy.

THERAPEUTIC IMPLICATIONS

Is the eosinophilia *per se* sometimes harmful to the organism and therefore requiring an active therapeutical approach? As noted above, the

hypereosinophilic syndrome is only one condition we know of where the disease manifestations may be a direct consequence of the eosinophilia. Theoretically, other conditions with sustained high eosinophilia as parasite infestations could be harmful too as a side effect of active eosinophil involvement. An increased frequency of cardiac disease has actually been described in tropical eosinophilia (38).

We do not know the mechanisms responsible for the disease manifestation of high eosinophilia, but as noted ECP is one possible candidate due to its abundance in the eosinophil granules and perhaps due to its effects on coagulation and the kallikrein-kinin systems (72). Very high levels of ECP in serum (4–10 mg/l) have actually been found in patients with the hypereosinophilic syndrome (unpublished). The therapeutic approach could be directed against the production of the eosinophils by the use of cytotoxic drugs. Successful treatment has also been described by reducing the eosinophil count by means of leukapheresis (28). High doses of corticosteroids is the most widely used therapy although the eosinophils of some patients with the hypereosinophilic syndrome seem to be remarkably resistant to this regime (15) in spite of the effect of cortison on normal eosinophils.

If ECP turns out to be involved in the destructive process of high eosinophilia, heparin should perhaps be given as it neutralizes ECP. One could speculate that complex formation with heparin would enhance the elimination rate of ECP as is found with other heparin complexes (23).

It is also possible that the "useful" aspect of eosinophil function could be exploited therapeutically. Pharmacological manipulation might be able to increase the accumulation of these cells, e.g. at the site of parasite invasion. The use of synthetic chemotactic tetrapeptides and modulation of the beta-adrenergic receptors come to mind. Identification of parasite-destroying eosinophil constituents may also have therapeutic implications.

However, the most important thing is still to

try to understand more of the mystery of the eosinophil function before rational therapeutic implications can be deduced.

CONCLUSIONS

The existence of the eosinophil granulocyte has been known for at least 100 years but its function remains mysterious. However, we have learned many new things about eosinophil production and eosinophil composition. Raised numbers of blood eosinophils are seen predominantly in allergic disease and in patients with parasite infestations. Most theories on eosinophil function have therefore been related to these conditions and have given the eosinophil the roles of 1) a modulator of the allergic reaction and 2) a parasite killer. Both suggestions have received some support by recent work, but none of the properties mentioned seems to be associated exclusively with the eosinophil.

Mast cell products, especially ECF-A, mediate local accumulation of eosinophils. Both ECF-A and histamine have been shown to enhance or unmask complement receptors on eosinophils. Eosinophils armed or "activated" in such a way can perhaps attack opsonized parasites more effectively.

The isolation of the major constituent of the human eosinophil granules, eosinophil cationic protein (ECP), has provided a new tool for studies on the function of this cell. The action of ECP can be involved both in the regulation of the humoral part of inflammation, by modulation of coagulation, fibrinolysis and kallikrein-kinin systems, and in killing of parasites. In addition detection of serum-ECP by a radioimmunoassay may provide useful information on the mechanisms of allergic reactions.

The activation of the eosinophil granulocyte is not always beneficial to the host. It may turn out that, especially in the high eosinophilia syndromes but also in diseases like asthma, the eosinophil may aggravate tissue destruction. It

therefore seems justified not to regard the eosinophil as the "good" cell in all situations. Clarification of the mechanisms behind eosinophil-mediated damage may lead to therapeutic implications. Our hypothesis, which has not yet been possible to test empirically, is that the major eosinophil constituent, ECP, may be responsible for the cardiac damage in high eosinophilia.

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