

REVIEW

Granules of human eosinophilic leucocytes and their mobilization

A. EGESTEN*, J. CALAFAT‡, H. JANSSEN‡, E. F. KNOL§, J. MALM† and T. PERSSON*

*Department of Medical Microbiology, and †Clinical Chemistry, Lund University, Malmö University Hospital, Malmö, Sweden, ‡Department of Cell Biology, the Netherlands Cancer Institute, Amsterdam, and §Department of Dermatology, University Medical Center, Utrecht, the Netherlands

Introduction

Paul Ehrlich was the first to observe a blood cell with a unique affinity for acidic dyes. He presented his discovery of the staining properties of eosinophils to the Berlin Physiological Society on January 17, 1879 [1]. The affinity for acid dyes has since been explained by the high content of cationic proteins within the cytoplasmic granules of the cell [1]. Although a long time has passed since eosinophils were discovered and much effort has been invested in studying them, their roles and functions are still not settled, and remain less clear than is the case, for example, for their relative in the myeloid series of differentiation, the neutrophil. Although associated with diseases such as parasitic infestation and asthma, depletion of eosinophils in these conditions does not necessarily have clear-cut effects on the course of the diseases [2,3]. Nor is a role for eosinophils clear in protection against cancer [4,5].

Since the cytoplasmic granules of eosinophils and their contents are such distinctive features of this cell, it is likely that many of its functions can be explained by the study of the granules and their release. The purpose of this review is to discuss the granule populations of eosinophils and mechanisms involved in their mobilization.

Terminology with respect to eosinophil granule populations

The terminology used for the different populations of eosinophil granules is not settled. The following terminology (Fig. 1) will be used in this review:

(a) Primary granules: the core-less, Charcot-Leyden crystal (CLC) protein-containing granules of immature and mature eosinophils. In some of the literature the term

‘primary granules’ is applied to the immature core-less granules of eosinophil promyelocytes, that later become the core-containing specific granules of mature eosinophils.

(b) Specific granules: the core-containing granules that are present in mature eosinophils. In eosinophil progenitors these are core-less and, in this review, will be referred to as ‘immature specific granules’. In some of the literature, specific granules are referred to as ‘secondary granules’.

(c) Small-type granules: a population of granules characterized by arylsulphatase activity as detected by cytochemistry and electronmicroscopy.

(d) Secretory vesicles: small dumbbell-shaped structures on a cross-section, characterized by their albumin content. In some literature they are referred to as ‘microgranules’ or ‘tubulovesicular structures’.

Transcriptional regulation of the genes for eosinophil granule proteins

Eosinophils originate from pluripotent stem cells in the bone marrow and the synthesis of granule proteins starts at the transition from eosinophilic myeloblast to promyelocyte [6]. Specific growth factors, especially interleukin (IL)-5, turn on distinct classes of genes, resulting in the characteristic phenotype of eosinophils [7]. DNA-binding proteins, e.g. transcription factors that coordinate the accessibility of RNA polymerase II to the promoter region of the gene, regulate transcription of the genes. Functional promoter regions of several proteins characteristic of eosinophils have been identified and their transcriptional regulation has been studied to varying extents.

At least five proteins are characteristic of the eosinophilic phenotype and are present in large quantities in the cell. These are major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and Charcot-Leyden crystal (CLC) protein.

Correspondence: Arne Egesten, MD, PhD, Department of Medical Microbiology, Entrance 78, Malmö University Hospital, SE-205 02 Malmö, Sweden. E-mail: Arne.Egesten@medforsk.mas.lu.se

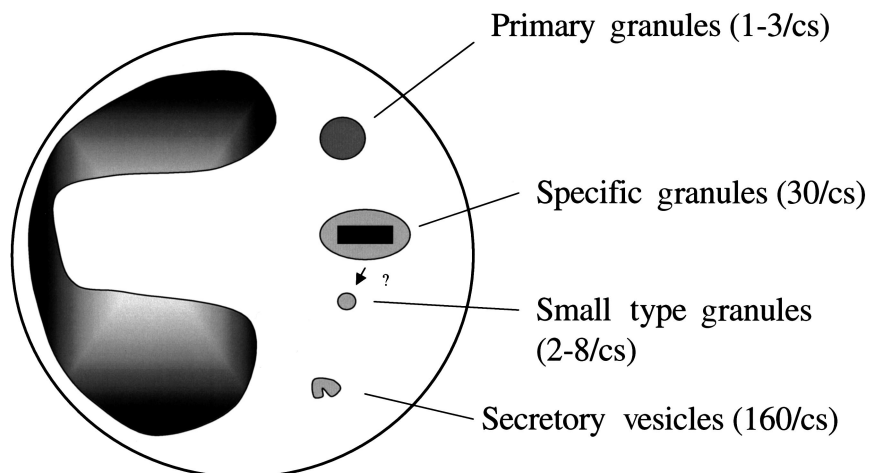


Fig. 1. Schematic drawing of the granule populations in a resting mature eosinophil. Primary granules are present at 1–3 granules per cross-section, specific granules at around 30 per cross-section, small-type granules at 2–8 per cross-section, and secretory vesicles at more than 160 per cross-section. Other compartments obviously also exist, transporting newly synthesized proteins or involved in the release of preformed proteins.

The gene encoding MBP is located on chromosome 11. It has two different promoters [8], generating two different transcripts of 1.0 and 1.6 kilo base pairs, respectively. The rationale for two promoter regions and the generation of two transcripts is not known. In leukaemic cell line HL60 cells the 1.0 transcript is the dominating one.

The EPO gene is located on chromosome 17, forming a cluster with genes for other peroxidases such as lactoperoxidase and myeloperoxidase [9]. The EPO gene consists of 12 exons and 11 introns [10]. 1.5 kilobase pairs of sequence upstream of the transcriptional start site and consensus binding sites for a number of positively and negatively acting elements in the promoter have been demonstrated [11].

The genes coding for the ribonucleases ECP and EDN are located on chromosome 14 [12] and belong to a family of primate ribonucleases [13]. Both genes consist of two exons and a single intron. The second exon contains the open reading frame. The intron is important for the transcriptional activity of these genes [14] and a consensus binding site for a transcription factor belonging to the NFAT-1 family seems to be of particular importance [15]. In addition, a consensus binding site for PU.1, a member of the ets family of genes, is present in the intron, and seems important for the transcriptional activity of the EDN gene [16].

The gene encoding CLC protein is located on chromosome 19 [12]. The CLC protein promoter sequence contains two consensus GATA binding sites, a purine-rich sequence that presents potential binding sites for PU.1 as well as sequences described in other myeloid-specific promoters [17].

Although characteristic of eosinophils, several of the cationic proteins are expressed in other tissues as well. The protein that hitherto is believed to be the most lineage-specific for eosinophils is EPO. Relatively low amounts of

EDN are present in basophils [18] and low amounts of both EDN and ECP in neutrophils [19,20]. There are large quantities of MBP in the placenta [21] and in basophils [18]. CLC protein is present in basophils but not in mast cells [22]. Recently, an MBP homologue, expressed in eosinophils, was described [23]. The amount present in eosinophils is not yet known.

Eosinophil progenitors

Eosinophil promyelocytes are rich in synthetic machinery such as endoplasmic reticulum and Golgi [24]. Staining of these cells for peroxidase shows reaction product within all cisternae of the rough endoplasmic reticulum, including transitional elements, and the perinuclear cisternae, the Golgi complex, and smooth vesicles at the periphery of the Golgi complex, as well as in granules [24]. Patch-clamp capacitance studies quantitating the size of granules that are incorporated into the plasma membrane upon degranulation have been performed on cord blood-derived eosinophils. These show that individual granules differ in size by multiples of unit vesicles, suggesting that the specific granules of eosinophils are formed by fusion of smaller unit vesicles [25]. Eosinophil promyelocytes contain abundant, large, round, core-less granules (Fig. 2a). The vast majority of these are immature specific granules holding cationic proteins, including MBP, the ribonucleases ECP and EDN, and EPO [26]. In a cross-section, a few primary granules, holding CLC protein, are also visible [27]. During maturation through the myelocyte and metamyelocyte stages of differentiation, the ER and Golgi gradually decrease, while most of the granules condense and become core-containing specific granules [24] (Fig. 2b).

Mature eosinophils contain secretory vesicles, which are not present in early bone marrow progenitors. These start to appear at terminal stages of differentiation, at the

myelocyte or metamyelocyte stage [28] and in a study of cord blood-derived eosinophils, their appearance coincided with a decrease in plasma membrane area, suggesting that the secretory vesicles are endocytic in origin [25].

Charcot-Leyden crystal protein

CLC protein is the sole component of the typical pyramidal, hexagonal crystals seen at sites involved in allergic inflammation (Fig. 3) [29]. It is a characteristic, although not unique, constituent of eosinophils, constituting 7–10% of the total protein content [30]. According to its genomic organization, it belongs to a family of galactose-binding lectins-galectins and is also named galectin-10 [31,32]. CLC protein possesses weak lysophospholipase activity but does not share homology with other characterized lysophospholipases [33]. Recently, the carbohydrate-binding properties of this protein were investigated and CLC protein did not show any affinity for β -galactosides and was found to bind mannose in a manner different from that of other related galectins [32].

CLC protein has no known cleaved or uncleaved signal peptide to direct it into the endoplasmic reticulum for further processing in the Golgi and sorting into a granular compartment during its biosynthesis [33]. Despite this, it is to be found in a subpopulation of granules; the primary granules (Fig. 3b) [34]. However, the main portion of CLC protein is probably extragranular, being localized to the cytoplasm and the euchromatin of the nucleus (Fig. 3a) [27]. In support of this, no lysophospholipase was found in the granule fraction, but in the membrane fraction upon subcellular fractionation [35]. Since it makes up such a large proportion of the total protein in the cell, it is also unlikely that the few primary granules can hold the entire cellular content of CLC protein. The dual localization (i.e. both intra- and extragranular) is most likely primary, but an alternative explanation may be that redistribution from the primary granules occurs after cellular activation. A possible explanation for the presence of CLC protein in both granules and cytoplasm/nucleus is an inefficient routing into the secretory pathway. Such inefficient routing has been demonstrated for other proteins, such as plasminogen activator inhibitor II [36].

In any case, CLC protein is enigmatic with respect to processing and sorting in the cell. Granule-bound CLC protein may be subjected to regulated release, while extragranular CLC protein may be subjected to non-classical release. The lysophospholipase activity of CLC protein may explain its extragranular localization. Activated eosinophils have a high production of lipid-derived proinflammatory agents, and derivatives from their synthesis may be harmful to the cell unless they are inactivated by a lysophospholipase. Intracellular CLC protein may

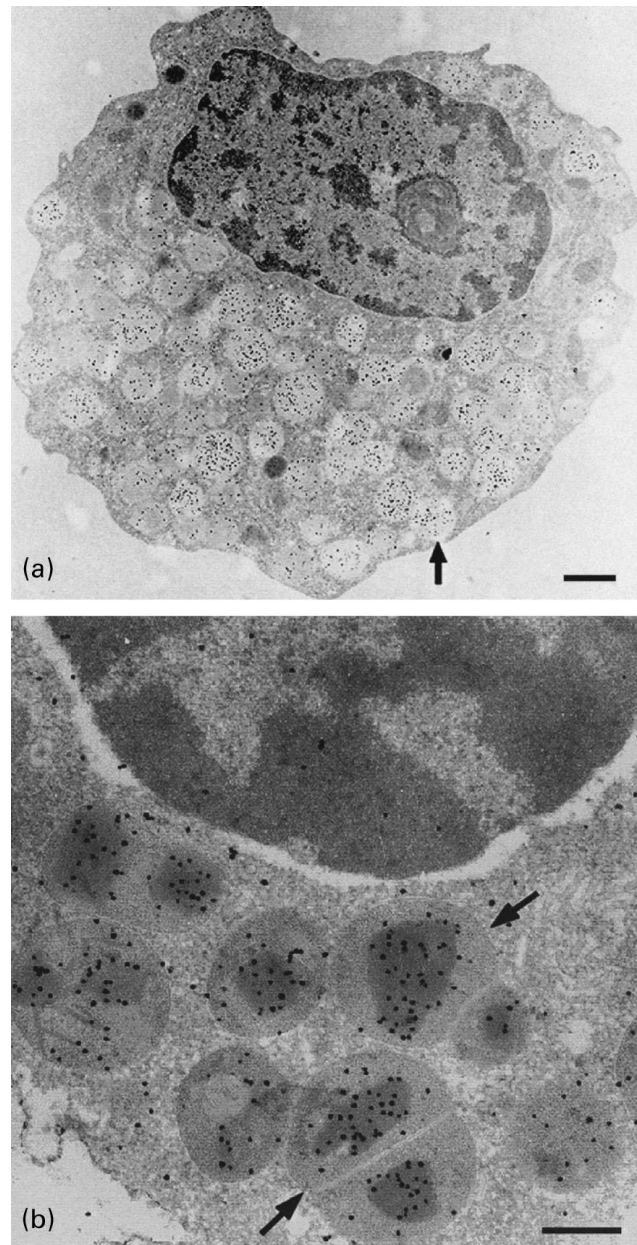


Fig. 2. (a) Electron micrograph showing an eosinophil promyelocyte of the bone marrow. Numerous immature specific granules, without crystalloid cores, are seen within the cytoplasm (arrow). The presence of ECP within the granules is seen as scattered black dots within the granules, the result of immunogold staining. From reference [26] with permission. (b) Core-containing specific granules in a mature eosinophil (arrows). The presence of MBP in the crystalloid core is shown by immunogold staining. This population of granules also contain ECP, EPO, EDN and several preformed cytokines.

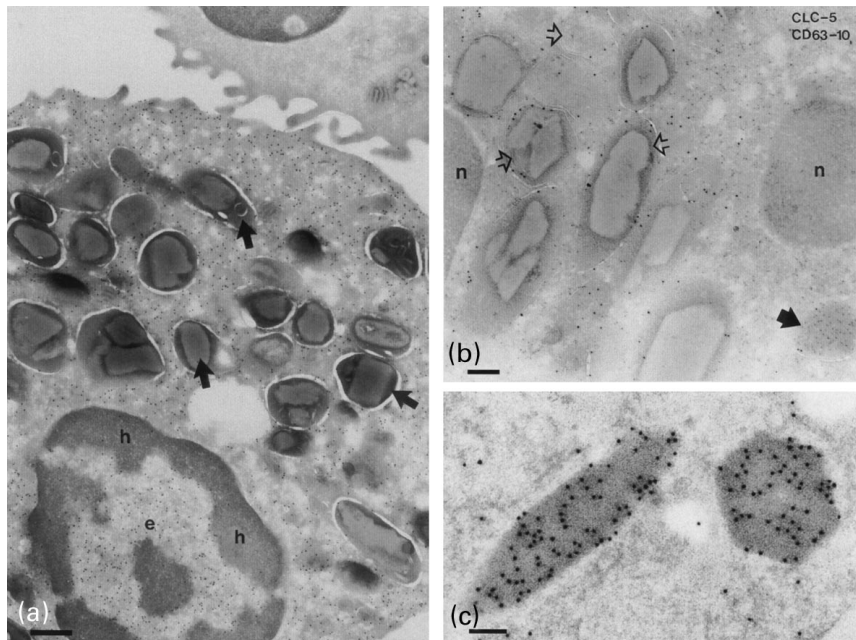


Fig. 3. (a) CLC protein distribution in a mature eosinophil, as detected by immunogold technique. The protein is present within the cytoplasm and the euchromatin (e) of the nucleus. The heterochromatin (h) and the specific granules (arrows) do not contain any CLC-protein. (b) In this electron micrograph, double-staining for the membrane-anchored tetraspanine CD63 and CLC-protein has been performed using immunogold technique. A primary granule is seen in the bottom right-hand corner, holding CLC protein (closed arrow). CD63 is present in the membrane of specific granules (open arrows). CLC protein is also present in the cytoplasm. (c) Charcot-Leyden crystals with their typical bipyramidal, hexagonal shape seen within the cytoplasm of a tissue-residing macrophage. The CLC protein detected by immunogold technique, is visualized as gold particles shattered over the surface of the crystals.

therefore have a protective role. However, at present, the role(s) for this protein that is present in such abundance in human eosinophils is definitely not clear.

Sequential expression of eosinophil granule proteins?

There is accumulating evidence that, in neutrophils, the different subpopulations of granules are formed as a consequence of sequential gene expression of the granule proteins [37], i.e. 'sorting by timing'. This results in a much greater heterogeneity of neutrophil granules than was previously postulated [38]. The importance of this may be to keep proteins with different functions separated, thus preventing interactions such as degradation, premature activation, or inactivation during storage. It can also allow sequential release of different proteins.

Could this hypothesis also apply to eosinophils? CLC protein is present in the primary granules of eosinophils [34] while the cationic proteins MBP, ECP, EDN, and EPO are present in the crystalloid-containing specific granules [39].

Investigation of possible sequential gene expression of the proteins characteristic of eosinophils has been performed in CD34⁺ cells isolated from peripheral blood and induced to differentiate towards the eosinophil lineage [40]. Early expression was seen in the case of CLC protein and could be detected by RT-PCR after a few days. Some overlap in the synthesis is suggested by the finding of EPO in some primary granules and CLC protein in some specific granules, as detected by immunoelectron microscopy [27]. Taken

together, this could indicate that CLC protein is synthesized before the cationic proteins, explaining the presence of CLC protein in a distinct granular compartment.

Specific granules

Most granule proteins are synthesized at the promyelocytic stage of differentiation [24]. In these cells, large, round core-less granules holding MBP, ECP, and EPO are present [26]. It is likely that these granules undergo condensation and later become the crystalloid-containing specific granules of mature eosinophils. In support of this, the precursor form of MBP (proMBP) is present in the large, round core-less granules, but immunoreactivity for this proform gradually decreases while the MBP-containing core appears during apparent condensation of the granules [41]. In the mature specific granules, MBP makes up the crystalloid core and the other proteins are present in the surrounding matrix of the granules (Fig. 2b) [39]. The specific granules also contain several preformed cytokines (Table 1). *In vitro* maturation of cord blood-derived CD34⁺ progenitors suggest that eosinophils express and store cytokines simultaneously with the cationic granule proteins during maturation [42]. The access to preformed cytokines in addition to *de novo* synthesis can be an advantage at sites of inflammation, since preformed cytokines are immediately available at larger quantities. Interestingly, despite the fact that a plethora of cytokines with diverse biological effects are stored in the same

Table 1. Cytokines, chemokines, and growth factors expressed by eosinophils. The cases where preformed protein have been detected in granules are indicated

Cytokine/chemokine	Presence in granules	Reference
IL-1 α		55
IL-2	SG	129
IL-3		130
IL-4	SG	131
IL-5	SG	132
IL-6	SG	133, 134
IL-8		135
IL-10		136
IL-12		137
IL-16		138
GM-CSF	SG	130, 139
TNF- α	SG	140
TGF- α	SG, V	52, 128
TGF- β 1		129
PDGF-b		141
VEGF		142
MCP-1		143
MIP-1 α		140
RANTES	SG, V	138, 144
Eotaxin	SG	145

Table 2. Granule proteins of human eosinophils

Protein	Localization	Comment	Reference
Major Basic Protein (MBP)	SG		39
MBP homologue	n.d.		23
Eosinophil Cationic Protein (ECP)	SG	ribonuclease	39
Eosinophil-Derived Neurotoxin (EDN)	SG	ribonuclease	146
Eosinophil Peroxidase (EPO)	SG		39
Charcot Leyden Crystal (CILC)-protein	PG, nucleus, cytoplasm	weak lysophospholipase, carbohydrate-binding properties	34, 27
Lysophospholipase (pancreatic type)	SG		147
Acid phosphatase	SG		49
Arylsulphatase B	SG, STG		45, 48
Phospholipase A2 (secretory)	SG	antibacterial	148
Bactericidal/Permeability-Increasing Protein (BPI)	SG	LPS-binding, antibacterial	149
NAMLAA	SG	antibacterial	150
Cytochrome b558	SV	part of NADPH-oxidase	52
FAD	SG		151
Catalase	SG		152
Urokinase	SG		153
CD63	SG	tetraspanin	27
CR3	SV	β 2-integrin	51
Proteoglycan	SG		154, 155
Alpha-1 antitrypsin	SG		156

Primary granules (PG), specific granules (SG), vesicles (V), not determined (n.d.).

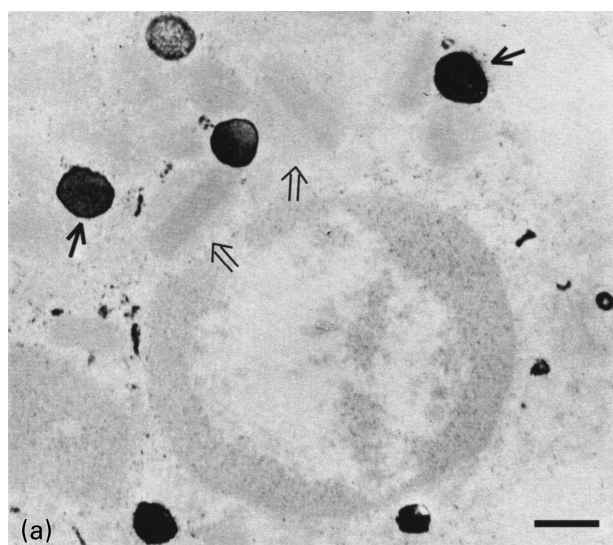
compartment (e.g. specific granules), selective release may occur after cellular stimulation [43].

Lysosome integral membrane protein (LIMP)-1 (CD63) is the only known membrane-anchored protein of specific granules [27]. It is a member of the transmembrane 4 superfamily (also called tetraspanins), that has been shown to form complexes with integrin receptors, and may participate in cell migration, respiratory burst and degranulation [44].

It should be emphasized that the four cationic proteins probably constitute a major part of the content of the specific granules. Many other proteins have been detected in various amounts, but their biological relevance should be evaluated with regard to their affinity for and effects on their targets (Table 2).

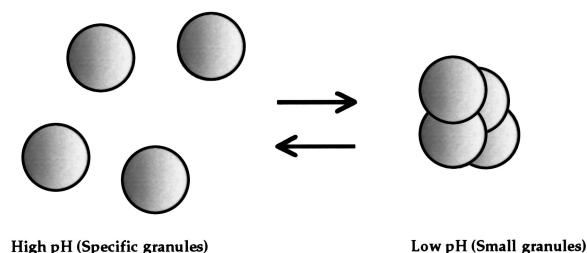
Small-type granules

In the mid-seventies, Parmley and Spicer described a population of small-type granules containing arylsulphatase activity (Fig. 4a) [45]. Arylsulphatase B (AsB), a lysosomal hydrolase that can degrade glycosaminoglycans (e.g. dermatan-sulphate and chondroitin-4-sulphate), has been considered a specific marker for this population of



(a) Low enzymatic activity

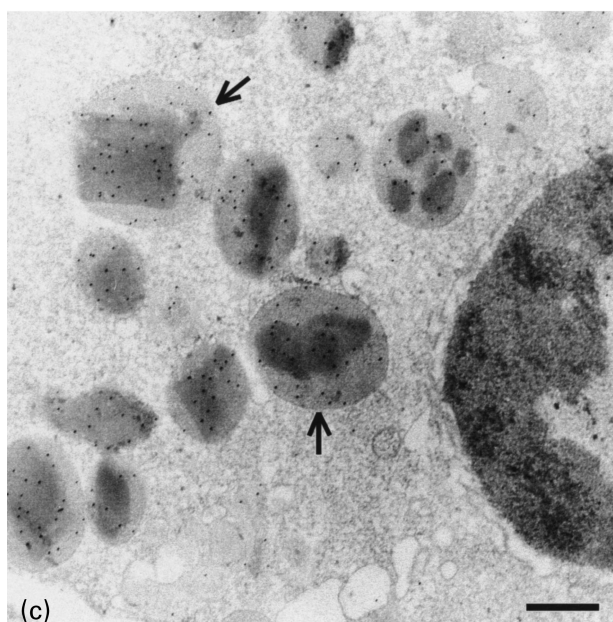
High enzymatic activity



High pH (Specific granules)

Low pH (Small granules)

(b)



(c)

granules. On a cross-section of a cell, 6–8 of these granules are visible, and their number increases in eosinophils at sites of activation and possible degranulation [45]. However, these small-type granules may be derived from the specific granules and several lines of evidence suggest such a relationship. AsB is an associating-dissociating enzyme; at high pH it has low enzymatic activity whereas at low pH it has high enzymatic activity [46] (Fig. 4b); therefore AsB may be present in a latent form in the specific granules. In addition, upon subcellular fractionation, the fraction containing specific granules was a rich source of AsB [47] and using immunogold technique, AsB immunoreactivity was detectable in specific granules [48] (Fig. 4c). Therefore, it can be hypothesized that small-type granules represent an activated compartment, containing enzymatically active AsB, whereas in specific granules, AsB is present in an enzymatically inactive form. Specific and small-type granules may be related and AsB is not an exclusive marker for the release of content from small-type granules.

Another protein that is present in an enzymatically inactive form in the specific granules is acid phosphatase. Enzymatic activity can be seen after cellular activation [49].

Secretory vesicles

Eosinophils also have a large number of secretory vesicles in their cytoplasm [50,51] (Fig. 5a). On a cross-section of a cell, 150–180 of these vesicles are visible. They contain membrane-bound cytochrome b558 (phox-22) and CD11b; the alpha-chain of the heterodimeric complement-receptor 3 (CR3) has also been localized to this population of organelles [52]. What is more, they contain albumin, indicating that they are endocytic in origin. However, in neutrophils, secretory vesicles are not part of a continuous pinocytosis and once mobilized they are not reformed [38]. The secretory vesicles of eosinophils may represent a compartment-holding membrane-bound receptors and other proteins that can be rapidly mobilized after cellular activation.

Fig. 4. (a) Electron micrograph showing small-type granules exhibiting arylsulphatase activity (appears in black; arrows), while specific granules lack such activity (pale, core-containing; open arrows). From reference [45] with permission. (b) Arylsulphatase B is an associating/dissociating enzyme. Enzymatically active tetramers are formed at low pH, while the enzyme subunits dissociate at higher pH and the enzymatic activity disappears. (c) Electron micrograph showing the presence of immunoreactive arylsulphatase B in specific granules as visualized by antibodies to AsB, detected by protein A attached to colloidal gold (arrows). From reference [48] with permission.

Additional compartments

Eosinophils also contain multilaminar bodies (MLBs) (Fig. 5b) [53]. In B cells, this structure has been shown to represent a late endocytic multilaminar structure with lysosomal characteristics, transporting major histocompatibility complex class II (MHC-II) molecules to the cell surface [54]. In eosinophils, MLBs contain transforming growth factor (TGF)- α [53]. Eosinophils can express MHC-II after activation [55], but the possible association between MHC-II and MLB has not been studied in eosinophils. In addition, TGF- α has been detected in a vesicular compartment of the cytoplasm. In double-labelling experiments, this latter compartment did not show the presence of CD63, a marker for lysosomes, nor with albumin of secretory vesicles [53]. Therefore, one may speculate upon the presence of other vesicular compartments, for example, participating in the constitutive release of *de novo* synthesized proteins.

Degranulation

Research in recent years has shown that eosinophils enter, and are transported in the bloodstream in a resting state, and become activated after encountering activated endothelium at sites of inflammation [56]. A small fraction of eosinophils seem to have a higher state of activation already in the bloodstream [57]. During some conditions, for example the idiopathic hypereosinophilic syndrome (HES), an increased activation of blood eosinophils is seen [58]. Many earlier studies of eosinophil functions were performed using resting cells, isolated from peripheral blood. However, after stimulation with cytokines *in vitro*, eosinophils are activated and become, for example, efficient phagocytes [59], and can increase their release of granule proteins [60].

The release of proteins can be constitutive, where newly synthesized proteins are continuously released. Constitutive

release is possible in eosinophils, for example the cytokines and chemokines expressed by tissue eosinophils (Table 1). Regulated release (degranulation) in response to specific stimuli takes place for the preformed granule proteins that are stored in the specific granules. Degranulation can take place into a phagosome/phagolysosome, containing an internalized microbe, within the cytoplasm of the cell (Fig. 6). The presence of ECP and EPO within phagosomes of eosinophils, indicates that specific granules fuse with phagosomes [61] (Fig. 6b). The cell can also release its granules onto a non-phagocytosable surface, such as a multicellular parasite, a form of release often referred to as 'frustrated phagocytosis' (Fig. 7).

In recent years, non-classical ways for eosinophil degranulation have been suggested.

These include so-called piecemeal degranulation [62] and cytolytic degranulation (e.g. necrosis) [63]. Evidence for piecemeal degranulation in eosinophils is based on observations of morphological alterations of granules and a change in distribution of granule proteins in activated eosinophils. In piecemeal degranulation, vesicles containing granule proteins seem to bud off from the specific granules [62]. Possibly, the small-type granules, which increase in number upon cellular activation and contain AsB that is also present in the specific granules, may be part of the same phenomenon [45]. The selective release of the CC chemokine RANTES after cellular stimulation with interferon- γ has also been attributed to piecemeal degranulation [43].

Cytolytic degranulation is a term applied to the free granules seen in association with necrotic eosinophils. This phenomenon seems to be present in atopic dermatitis [63] and allergic inflammation of the upper airways of humans [64]. Eosinophil necrosis is also apparent after IgG-induced activation *in vitro* [65]. Whether cytolytic degranulation is a regulated process or a result of eosinophils succumbing due to environmental factors is not known.

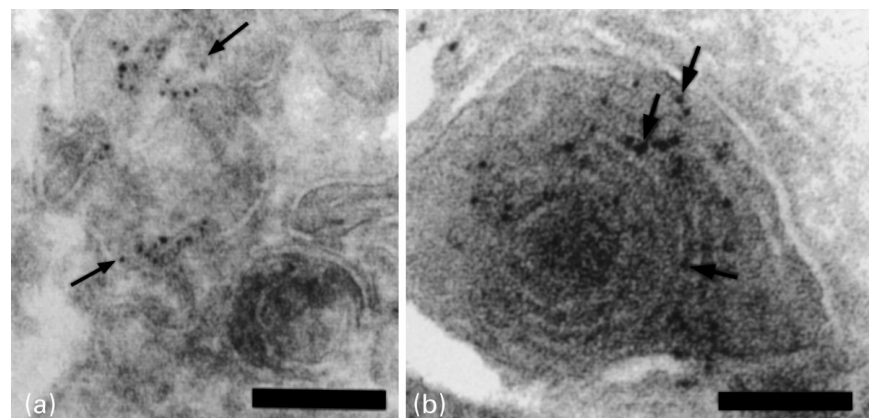


Fig. 5. (a) Secretory vesicles, sometimes referred to as microgranules, are small dumbbell-shaped structures abundantly present in the cytoplasm of eosinophils. They contain albumin, whose presence is indicated by immunogold staining in this electron micrograph (arrows). Bar = 500 nm. (b) A multilaminar body in the cytoplasm of an eosinophil. Immunogold detection of TGF- α is seen (arrows). Bar = 500 nm.

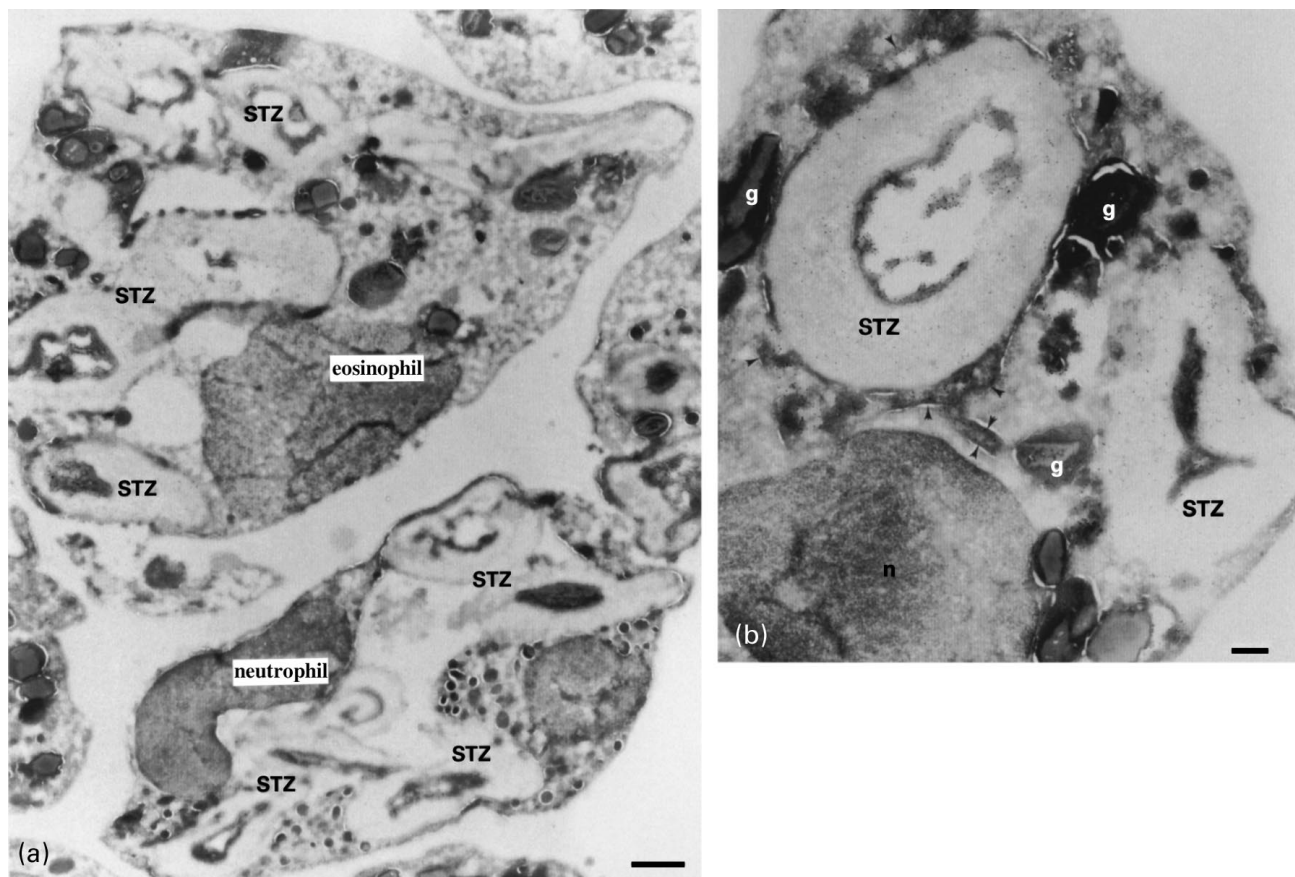


Fig. 6. (a) Eosinophil and neutrophil, showing extensive uptake of serum-treated zymosan (STZ) into phagosomes/phagolysosomes. (b) This picture shows degranulation into a zymosan-containing phagosome (phagolysosome). Specific granules (g) appear to fuse with and release their contents into the phagosome containing STZ (arrowheads). EPO is detected within the phagosome by immunogold and is seen as small dots (n = nucleus).

Receptors and ligands

Specific receptors on the surface of eosinophils can recognize the target, via, for example, immunoglobulins and complement products that are bound to the surface of the target. Cross-linking of the receptors through binding to immobilized ligand can induce strong signals for phagocytosis [59] and degranulation [66] *in vitro*. It is reasonable to believe that immobilized ligands help direct eosinophil degranulation towards the target. The receptors that so far are best recognized for their ability to induce degranulation will be discussed here.

Complement Receptor 3

The immobilized complement fragment *i*C3b, albumin, factor X, and fibrinogen all bind to CR3 (also called CD11b/CD18, α_m/β_2 , or Mac-1), which is present on the surface of eosinophils. Activation and cross-linking of this receptor induces a strong signal for degranulation in eosinophils [67].

Cellular activation can increase the affinity of CR3 for its ligand. This has been elegantly illustrated: eosinophils isolated from the peripheral blood of healthy donors only bind serum-treated zymosan to a low extent; however, stimulation of eosinophils with cytokines such as GM-CSF, IL-3, IL-5 [59], TNF- α [61], or platelet-activating factor (PAF) [68] causes a rapid increase in eosinophil binding and phagocytosis of serum-treated zymosan.

Apart from external stimuli, eosinophils themselves can, upon encounter with a serum-opsonized target, bind via CR3, which elicits production of PAF. This can increase the activation of the cell itself, and adjacent cells, via auto- and paracrine loops, and enhance degranulation [69,70].

The importance of CR3 in eosinophil degranulation is also illustrated by the fact that cross-linking of CR3 by antibodies results in degranulation [71].

Monoclonal antibodies against CR3 inhibit IgG-induced eosinophil degranulation [72], and it has been suggested that Fc-receptors for IgG and CR3 are physically associated with each other [73].

Receptors for IgA

Immobilized IgA, especially secretory IgA (sIgA) induces strong signals for degranulation in eosinophils via Fc α R [74]. Also, the isolated secretory component of sIgA

induces degranulation in eosinophils [75], but not in neutrophils [76]. This further points to a role for eosinophils in host defence at mucosal barriers, where sIgA is present in high concentrations.

Receptors for IgG

Immobilized IgG is a strong inducer of eosinophil degranulation and eosinophils have a high density of the low-affinity Fc-receptor for IgG (Fc γ RII, CD32) on their surface [77].

The binding of complexed IgG by eosinophils increases after stimulation of the cells by granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-5 [78] and these cytokines also enhance IgG-induced degranulation [60].

A minor population of resting eosinophils has surface expression of the high affinity Fc-receptor for IgG (CD16, Fc γ RI) and upon stimulation by chemoattractants, a rapid, but transient surface expression of this receptor can be induced from an intracellular pool [79]. Distinctive roles for this receptor in eosinophil degranulation have not been investigated.

Receptors for IgE

Both eosinophilia and high levels of IgE are typical components of allergic inflammation. Therefore, it seems reasonable that eosinophils should have the capacity to interact with IgE. An interesting observation was that selective release of EDN occurred after stimulation of eosinophils with complexed IgE [80]. However, the importance of IgE in eosinophil immunobiology has been a matter of controversy [77]. The presence of high-affinity receptors for IgE (Fc ϵ RI), has been reported in patients with the idiopathic hypereosinophilic syndrome [81]. Also, other IgE-binding structures, such as the low-affinity receptors for IgE (CD23, Fc ϵ RII) and Mac-2/ ϵ BL have been reported to be present on eosinophils [82]. Recently, intracellular expression and release of the α -chain of Fc ϵ RI was reported in eosinophils, but little or no Fc ϵ RI or CD23 could be detected on the cell surface of eosinophils from normal donors or patients with severe eosinophilia

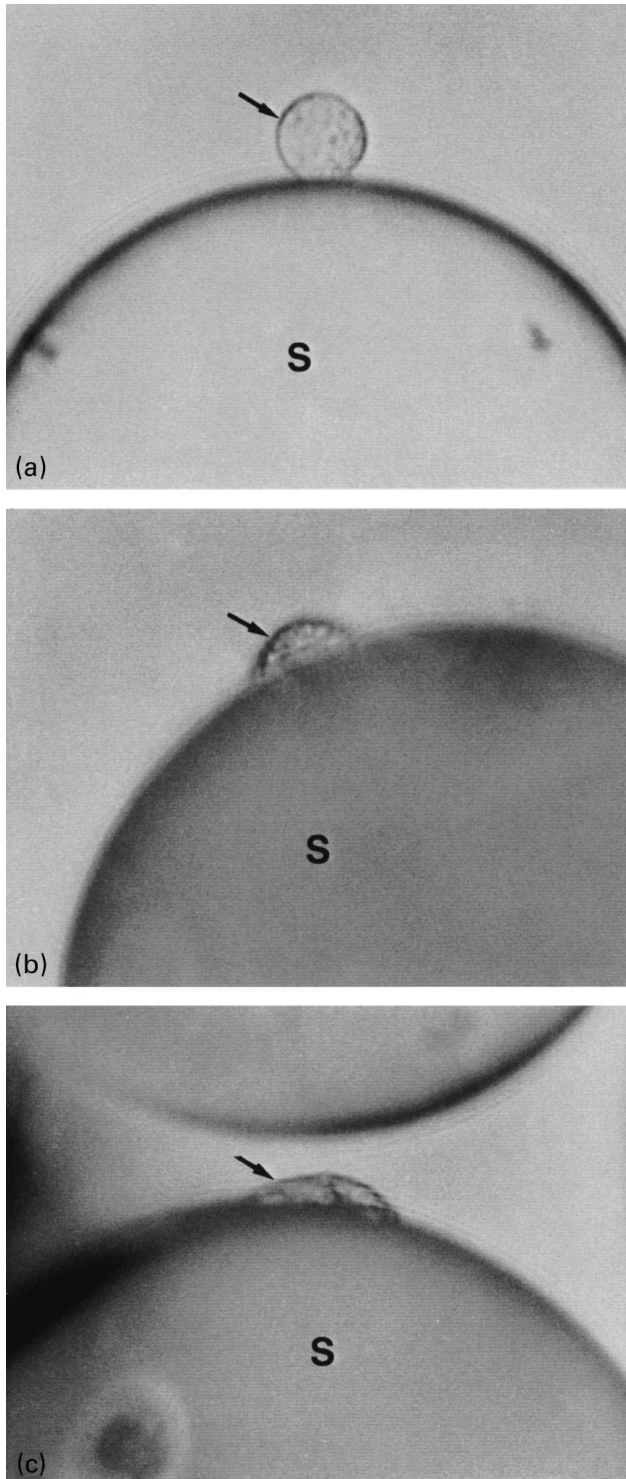


Fig. 7. Eosinophil encounter with serum-treated Sephadex bead (S) representing a semiphysiological parasite model. (a) The cells first appear round, but upon binding by CR3, the cells become flattened due to interactions with the cytoskeleton (b and c). Degranulation occurs onto the surface of the non-phagocytosable object and granule proteins typical of eosinophils can be detected in the surrounding medium after this 'frustrated phagocytosis'. From reference [65] with permission.

[77,83]. Nor did eosinophils respond with detectable degranulation upon challenge with human IgE or anti-human IgE [77].

Other receptors for complement and immunoglobulins

Eosinophils possess receptors for the complement fragment C1q and C5a and they enhance IgG- or iC3b-induced degranulation, but does not by themselves cause significant release of specific granule content [79,84,85]. A receptor for the soluble complement fragment C3a is present on the surface of eosinophils [86] and causes granule release in cells that have been pretreated with cytochalasin B, resulting in disruption of the cytoskeleton [87].

On the basis of binding experiments, no significant amounts of receptors for IgM or IgD seem to be present on eosinophils [88].

Tetraspanins

Eosinophils isolated from peripheral blood express the tetraspanins CD9, CD37, CD53 (MRC OX44), CD63 (LIMP-1) and CD81 (TAPA-1) [44]. Eosinophil degranulation can be induced by cross-linking of CD9 by antibodies [89]. The release was abolished by pretreatment of the eosinophils with monoclonal antibodies against CD18, suggesting that $\beta 2$ integrins are involved in the response [89].

Soluble secretagogues

Many soluble stimulatory substances, such as PMA, IL-3, IL-5, GM-CSF, C5a, PAF, eotaxin, and fMLP, seem to have weak or no direct effects with respect to release of the contents of the specific granules from cells in suspension; rather, they enhance the release in response to immobilized immunoglobulins and complement (e.g. iC3b) [60,90]. Soluble stimuli may mobilize receptors to the cell surface (e.g. CR3) [91], and their affinity for the ligand may increase [59,68,78]. The increase in CR3 on the cell surface after stimulation, suggests that the secretory vesicles can be mobilized and their membrane integrated into the cell membrane. The contribution of CR3-mediated adhesion to eosinophil degranulation *in vitro* is difficult to determine, since also cells 'in suspension' may aggregate (homotypic aggregation) via CR3 [92], or adhere to the walls of a tube, possibly due to binding via CR3 to albumin coating the tube [93].

Interaction of eosinophil granules with the cell membrane in regulated exocytosis

Using patch-clamp technique, eosinophils have been found

to be capable of degranulation early during maturation, probably while the granules are only starting to form, at the transition from promyelocyte to myelocyte [25].

Studies on horse eosinophils, using GTP γ S and patch-clamp technique, have revealed three distinct fusion processes during eosinophil degranulation [94]. At low-grade stimulation, there seems to be release of single granules through fusion with the cell membrane, and formation of pores in the plasma membrane [95]. At higher doses of GTP γ S, granules fuse inside the cell, forming large compound granules which then fuse with the cell membrane (compound exocytosis) or granules fuse with a single granules that has formed a pore in the membrane (cumulative exocytosis) [95]. In support of these findings, activated eosinophils both *in vivo* and *in vitro* often show large vacuoles and pore formation [96,97]. The currently suggested routes for release of the contents of specific granules are shown in Fig. 8.

Proteins in the membrane of intracellular granules and vesicles interact with membrane-bound proteins in the cell membrane during exocytosis. The interaction between membrane-bound SNARE (soluble N-ethylmaleimide sensitive factor attachment receptor) proteins in cell secretion has been an area of extensive research in recent years [98]. However, little is known about the roles of these proteins in constitutive release and regulated exocytosis in eosinophils. Syntaxin-3 A (t-SNARE) is present in guinea-pig eosinophils [99].

Cytotoxic mechanisms

MBP and ECP may be the cause of damage to lipid bilayers, such as plasma membranes of target cells [100,101]. This may be due to their cationic nature. In the case of ECP, the formation of transmembrane pores and thereby damage to a target cell has been shown [100]. Both ECP and EDN, in particular the latter, possess ribonuclease activity [102] which may be important for anti-microbial activity. However, the bactericidal effect of ECP does not seem to be dependent on ribonuclease activity [103]. On the other hand, activity against the double-stranded RNA-virus respiratory syncytial virus (RSV) has been shown for both ECP and EDN, suggesting that the ribonuclease activity of these proteins can be important for host-defence functions in some contexts, e.g. infection by RNA viruses [104,105].

ECP and, in particular, EPO most likely participates in the formation of toxic compounds from halides and products from the NADPH oxidase system of eosinophils [106,107].

Possible targets for the cytotoxic effects

Eosinophilia of blood and tissue is classically associated

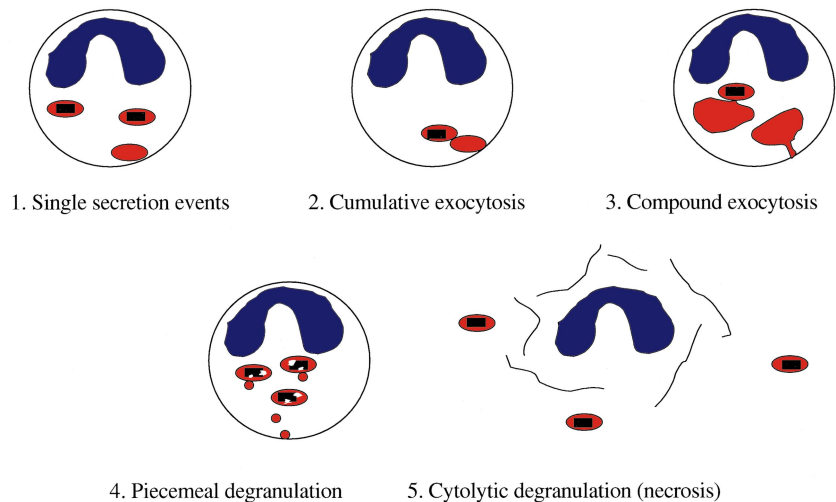


Fig. 8. Suggested routes for release of the content of specific granules.

with parasitic and especially invasive helminthic infections, such as filariasis and bilharziosis [108]. The cytotoxicity of eosinophil granule proteins against several nematodes and trematodes in their larval stage has been shown [109–113].

Increased levels of eosinophils in peripheral blood are not typical for either bacterial or viral infection. However, antibacterial activity has been demonstrated for isolated ECP, MBP [114], and EPO [115]. Therefore, eosinophils may fulfil antibacterial host-defence functions in tissues where they accumulate in larger numbers than are present in the circulation [116].

Infection of the airways by respiratory syncytial virus (RSV) induces airway inflammation involving eosinophils [117]. RSV is an enveloped double-stranded RNA virus and both ECP and EDN have antiviral effect against RSV *in vitro* [104,105].

Roles for eosinophils in host defence against neoplasia have been suggested and are supported by the finding that EPO is toxic to mammalian tumour cells [118]. The presence of eosinophils in colonic carcinoma is considered a favourable prognostic factor but it is not clear whether eosinophils are cytotoxic to cancer cells [4,5,119,120].

Other effects of released granule proteins

MBP is by far the best studied eosinophil granule protein for diverse biological effects, through the work of Gleich and coworkers. Apart from being toxic to parasites and tumour cells, MBP is toxic to a variety of host cells, including splenic, intestinal, and endothelial cells, and airway epithelium [121]. MBP has a deleterious effect on mammalian ciliary activity [122]. MBP, and to a lesser extent EPO, also function as endogenous allosteric inhibitors of agonist binding to the M2 muscarinic receptor [123]. Released EPO induces mast cell secretion [124].

Also, the plethora of preformed cytokines and chemokines present in eosinophil granules and expressed by mature eosinophils may have proinflammatory effects after release at sites of inflammation (Table 1). Taken together, several of the constituents of eosinophil granules may be of importance in explaining the bronchoconstriction, oedema, and tissue damage seen during allergic inflammation, for example in asthma.

Feared complications of long-standing severe eosinophilia are endomyocardial fibrosis and thromboembolic events [1,125]. Eosinophils can induce DNA synthesis in fibroblasts [126], and release of the growth factors TGF- α and TGF- β 1 may be of importance for the connective tissue remodelling seen in conjunction with eosinophilia [127,128]. Released cationic proteins may be of importance in the development of thrombosis in diseases such as HES, but the exact mechanisms are not yet clear.

Thus, the clearest role for the released granule proteins of eosinophils so far seems to be in the body's defence against helminthic infestation. They may also have unfavourable roles in diseases such as asthma.

References

- 1 Spry CJF. Eosinophils. A Comprehensive Review and Guide to the Scientific and Medical Literature. Oxford Medical Publications, Oxford, 1988.
- 2 Sher A, Coffman RL, Hieny S, Cheever AW. Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. *J Immunol* 1990; 145:3911–6.
- 3 Leckie MJ, ten Brinke A, Khan J et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; 356:2144–8.
- 4 Tepper RI, Coffman RL, Leder P. An eosinophil-dependent

- mechanism for the antitumor effect of interleukin-4. *Science* 1992; 257:548–51.
- 5 Kruger-Krasagakes S, Li W, Richter G, Diamantstein T, Blankenstein T. Eosinophils infiltrating interleukin-5 gene-transfected tumors do not suppress tumor growth. *Eur J Immunol* 1993; 23:992–5.
 - 6 Bainton DF. Developmental biology of neutrophils and eosinophils. In: Gallin JI, Snyderman R, eds. *Inflammation: Basic Principles and Clinical Correlates*. Philadelphia: Lippincott Williams & Wilkins, 1999: 61–76.
 - 7 Sanderson CJ. Interleukin-5, eosinophils, and disease. *Blood*, 1992; 79:3101–19.
 - 8 Li MS, Sun L, Satoh T, Fisher LM, Spry CJ. Human eosinophil major basic protein, a mediator of allergic inflammation, is expressed by alternative splicing from two promoters. *Biochem J* 1995; 305:921–7.
 - 9 Sakamaki K, Kanda N, Ueda T, Aikawa E, Nagata S. The eosinophil peroxidase gene forms a cluster with the genes for myeloperoxidase and lactoperoxidase on human chromosome 17. *Cytogenet Cell Genet* 2000; 88:246–8.
 - 10 Sakamaki K, Tomonaga M, Tsukui K, Nagata S. Molecular cloning and characterization of a chromosomal gene for human eosinophil peroxidase. *J Biol Chem* 1989; 264:16828–36.
 - 11 Yamaguchi Y, Zhang DE, Sun Z et al. Functional characterization of the promoter for the gene encoding human eosinophil peroxidase. *J Biol Chem* 1994; 269:19410–9.
 - 12 Mastrianni DM, Eddy RL, Rosenberg HF et al. Localization of the human eosinophil Charcot-Leyden crystal protein (lysophospholipase) gene (CLC) to chromosome 19 and the human ribonuclease 2 (eosinophil-derived neurotoxin) and ribonuclease 3 (eosinophil cationic protein) genes (RNS2 and RNS3) to chromosome 14. *Genomics* 1992; 13:240–2.
 - 13 Rosenberg HF, Dyer KD, Tiffany HL, Gonzalez M. Rapid evolution of a unique family of primate ribonuclease genes. *Nat Genet* 1995; 10:219–23.
 - 14 Tiffany HL, Handen JS, Rosenberg HF. Enhanced expression of the eosinophil-derived neurotoxin ribonuclease (RNS2) gene requires interaction between the promoter and intron. *J Biol Chem* 1996; 271:12387–93.
 - 15 Handen JS, Rosenberg HF. Intronic enhancer activity of the eosinophil-derived neurotoxin (RNS2) and eosinophil cationic protein (RNS3) genes is mediated by an NFAT-1 consensus binding sequence. *J Biol Chem* 1997; 272:1665–9.
 - 16 van Dijk TB, Caldenhoven E, Raaijmakers JA, Lammers JW, Koenderman L, de Groot RP. The role of transcription factor PU.1 in the activity of the intronic enhancer of the eosinophil-derived neurotoxin (RNS2) gene. *Blood* 1998; 91:2126–32.
 - 17 Gomolin HI, Yamaguchi Y, Paulpillai AV, Dvorak LA, Ackerman SJ, Tenen DG. Human eosinophil Charcot-Leyden crystal protein. cloning and characterization of a lysophospholipase gene promoter. *Blood* 1993; 82:1868–74.
 - 18 Abu-Ghazaleh RI, Dunnette SL, Loegering DA et al. Eosinophil granule proteins in peripheral blood granulocytes. *J Leukoc Biol* 1992; 52:611–8.
 - 19 Rosenberg HF, Tenen DG, Ackerman SJ. Molecular cloning of the human eosinophil-derived neurotoxin: a member of the ribonuclease gene family. *Proc Natl Acad Sci USA* 1989; 86:4460–4.
 - 20 Sur S, Glitz DG, Kita H et al. Localization of eosinophil-derived neurotoxin and eosinophil cationic protein in neutrophilic leukocytes. *J Leukoc Biol* 1998; 63:715–22.
 - 21 Maddox DE, Kephart GM, Coulam CB, Butterfield JH, Benirschke K, Gleich GJ. Localization of a molecule immunochemically similar to eosinophil major basic protein in human placenta. *J Exp Med* 1984; 160:29–41.
 - 22 Ackerman SJ, Weil GJ, Gleich GJ. Formation of Charcot-Leyden crystals by human basophils. *J Exp Med* 1982; 155:1597–609.
 - 23 Plager DA, Loegering DA, Weiler DA et al. A novel and highly divergent homolog of human eosinophil granule major basic protein. *J Biol Chem* 1999; 274:14464–73.
 - 24 Bainton DF, Farquhar MG. Segregation and packaging of granule enzymes in eosinophilic leukocytes. *J Cell Biol* 1970; 45:54–73.
 - 25 Scepke S, Lindau M. Exocytotic competence and intragranular fusion in cord blood-derived eosinophils during differentiation. *Blood* 1997; 89:510–7.
 - 26 Egesten A, Calafat J, Weller PF et al. Localization of granule proteins in human eosinophil bone marrow progenitors. *Int Arch Allergy Immunol* 1997; 114:130–8.
 - 27 Calafat J, Knol EF, Janssen H, Egesten A. Ultrastructural localization of Charcot-Leyden crystal protein in human eosinophils and basophils. *Eur J Haematol* 1997; 58:56–66.
 - 28 Zucker-Franklin D. Eosinophil structure and maturation. In: Mahmoud, AA, Austen, KF, Simon, AS *The eosinophil in health and disease*. New York, NY, Grune and Stratton, 1980.
 - 29 Weller PF, Bach D, Austen KF. Human eosinophil lysophospholipase. the sole protein component of Charcot-Leyden crystals. *J Immunol* 1982; 128:1346–9.
 - 30 Weller PF, Bach DS, Austen KF. Biochemical characterization of human eosinophil Charcot-Leyden crystal protein (lysophospholipase). *J Biol Chem* 1984; 259:15100–5.
 - 31 Dyer KD, Handen JS, Rosenberg HF. The genomic structure of the human Charcot-Leyden crystal protein gene is analogous to those of the galectin genes. *Genomics* 1997; 40:217–21.
 - 32 Swaminathan GJ, Leonidas DD, Savage MP, Ackerman SJ, Acharya KR. Selective recognition of mannose by the human eosinophil Charcot-Leyden crystal protein (galectin-10): a crystallographic study at 1.8: a resolution. *Biochem* 1999; 38:13837–43.
 - 33 Ackerman SJ, Corrette SE, Rosenberg HF et al. Molecular cloning and characterization of human eosinophil Charcot-Leyden crystal protein (lysophospholipase). Similarities to IgE binding proteins and the S-type animal lectin superfamily. *J Immunol* 1993; 150:456–68.
 - 34 Dvorak AM, Letourneau L, Login GR, Weller PF, Ackerman SJ. Ultrastructural localization of the Charcot-Leyden crystal protein (lysophospholipase) to a distinct crystalloid-free granule population in mature human eosinophils. *Blood* 1988; 72:150–8.

- 35 Weller PF, Goetzel EJ, Austen KF. Identification of human eosinophil lysophospholipase as the constituent of Charcot-Leyden crystals. *Proc Natl Acad Sci USA* 1980; 77:7440–3.
- 36 von Heijne G, Liljestrom P, Mikus P, Andersson H, Ny T. The efficiency of the uncleaved secretion signal in the plasminogen activator inhibitor type 2 protein can be enhanced by point mutations that increase its hydrophobicity. *J Biol Chem* 1991; 266:15240–3.
- 37 Cowland JB, Borregaard N. The individual regulation of granule protein mRNA levels during neutrophil maturation explains the heterogeneity of neutrophil granules. *J Leukoc Biol* 1999; 66:989–95.
- 38 Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* 1997; 89:3503–21.
- 39 Egesten A, Alumets J, von Mecklenburg C, Palmegren M, Olsson I. Localization of eosinophil cationic protein, major basic protein and eosinophil peroxidase by immunoelectron microscopic technique. *J Histochem Cytochem* 1986; 11:1399–403.
- 40 Rosenberg HF, Dyer KD, Li F. Characterization of eosinophils generated in vitro from CD34+ peripheral blood progenitor cells. *Exp Hematol* 1996; 24:888–93.
- 41 Popken-Harris P, Checkel J, Loegering D et al. Regulation and processing of a precursor form of eosinophil granule major basic protein (proMBP) in differentiating eosinophils. *Blood* 1998; 92:623–31.
- 42 Mahmudi-Azer S, Velasquez JR, Lacy P, Denburg JA, Moqbel R. Immunofluorescence analysis of cytokine and granule protein expression during eosinophil maturation from cord blood-derived CD34 progenitors. *J Allergy Clin Immunol* 2000; 105:1178–84.
- 43 Lacy P, Mahmudi-Azer S, Bablitz B et al. Rapid mobilization of intracellularly stored RANTES in response to interferon- γ in human eosinophils. *Blood* 1999; 94:23–32.
- 44 Matsumoto K, Bochner BS, Wakiguchi H, Kurashige T. Functional expression of transmembrane 4 superfamily molecules on human eosinophils. *Int Arch Allergy Immunol* 1999; 120 (Suppl. 1):38–44.
- 45 Parmley RT, Spicer SS. Cytochemical and ultrastructural identification of a small type granule in human late eosinophils. *Lab Invest* 1974; 30:557–67.
- 46 Weller PF, Austen KF. Human eosinophil arylsulfatase B. Structure and activity of the purified tetrameric lysosomal hydrolase. *J Clin Invest* 1983; 71:114–23.
- 47 Weller PF, Moynihan-Earley RA, Dvorak HF, Dvorak AM. Cytoplasmic lipid bodies of human eosinophils: subcellular isolation and analysis of arachidonate incorporation. *Am J Pathol* 1991; 138:141–8.
- 48 Egesten A, Weller PF, Olsson I. Arylsulfatase B is present in crystalloid-containing granules of human eosinophil granulocytes. *Int Arch Allergy Immunol* 1994; 104:207–10.
- 49 Bass DA, Lewis JC, Szejda P, Cowley L, McCall CE. Activation of lysosomal acid phosphatase of eosinophil leukocytes. *Lab Invest* 1981; 44:403–9.
- 50 Schaeffer HE, Hübner G, Fischer R. Spezifische Mikrogranula in Eosinophilen. *Acta Haemat* 1973; 50:92–104.
- 51 Ginsel LA, Onderwater JJ, Fransen JA, Verhoeven AJ, Roos D. Localization of the low-Mr subunit of cytochrome b558 in human blood phagocytes by immunoelectron microscopy. *Blood* 1990; 76:2105–16.
- 52 Calafat J, Kuijpers TW, Janssen H, Borregaard N, Verhoeven AJ, Roos D. Evidence for small intracellular vesicles in human blood phagocytes containing cytochrome b558 and the adhesion molecule CD11b/CD18. *Blood* 1993; 81:3122–9.
- 53 Egesten A, Calafat J, Knol EF, Janssen H, Walz TM. Subcellular localization of TGF- α in human eosinophil granulocytes. *Blood* 1996; 87:3910–8.
- 54 Calafat J, Nijenhuis M, Janssen H et al. Major histocompatibility complex class II molecules induce the formation of endocytic MIIC-like structures. *J Cell Biol* 1994; 126:967–77.
- 55 Weller PF, Rand TH, Barrett T, Elovic A, Wong DT, Finberg RW. Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 α expression. *J Immunol* 1993; 150:2554–62.
- 56 Bochner BS, Schleimer RP. The role of adhesion molecules in human eosinophil and basophil recruitment. *J Allergy Clin Immunol* 1994; 94:427–38.
- 57 Blom M, Tool AT, Mul FP, Knol EF, Roos D, Verhoeven AJ. Eosinophils isolated with two different methods show different characteristics of activation. *J Immunol Meth* 1995; 178:183–93.
- 58 Winqvist I, Olofsson T, Olsson I, Persson AM, Hallberg T. Altered density, metabolism and surface receptors of eosinophils in eosinophilia. *Immunology* 1982; 47:531–9.
- 59 Blom M, Tool AT, Kok PT, Koenderman L, Roos D, Verhoeven AJ. 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.
- 60 Fujisawa T, Abu-Ghazaleh R, Kita H, Sanderson CJ, Gleich GJ. Regulatory effect of cytokines on eosinophil degranulation. *J Immunol* 1990; 144:642–6.
- 61 Egesten A, Blom M, Calafat J, Janssen H, Knol EF. Eosinophil granulocyte interaction with serum-opsonized particles. binding and degranulation are enhanced by tumor necrosis factor α . *Int Arch Allergy Immunol* 1998; 115:121–8.
- 62 Dvorak AM, Furitsu T, Letourneau L, Ishizaka T, Ackerman SJ. Mature eosinophils stimulated to develop in human cord blood mononuclear cell cultures supplemented with recombinant human interleukin-5. Part I. Piecemeal degranulation of specific granules and distribution of Charcot-Leyden crystal protein. *Am J Pathol* 1991; 138:69–82.
- 63 Cheng JF, Ott NL, Peterson EA et al. Dermal eosinophils in atopic dermatitis undergo cytolytic degeneration. *J Allergy Clin Immunol* 1997; 99:683–92.
- 64 Erjefält JS, Greiff L, Andersson M et al. Allergen-induced eosinophil cytotoxicity is a primary mechanism for granule protein release in human upper airways. *Am J Respir Crit Care Med* 1999; 160:304–12.

- 65 Weiler CR, Kita H, Hukee M, Gleich GJ. Eosinophil viability during immunoglobulin-induced degranulation. *J Leukoc Biol* 1996; 60:493–501.
- 66 Winqvist I, Olofsson T, Olsson I. Mechanisms for eosinophil degranulation; release of the eosinophil cationic protein. *Immunology* 1984; 51:1–8.
- 67 Egesten A, Gullberg U, Olsson I, Richter J. Phorbol-ester-induced degranulation in adherent human eosinophil granulocytes is dependent on CD11/CD18-leukocyte integrins. *J Leukoc Biol* 1993; 53:287–93.
- 68 Blom M, Tool AT, Roos D, Verhoeven AJ. Priming of human eosinophils by platelet-activating factor enhances the number of cells able to bind and respond to opsonized particles. *J Immunol* 1992; 149:3672–7.
- 69 Tool AT, Koenderman L, Kok PT, Blom M, Roos D, Verhoeven AJ. Release of platelet-activating factor is important for the respiratory burst induced in human eosinophils by opsonized particles. *Blood* 1992; 79:2729–32.
- 70 Bartemes KR, McKinney S, Gleich GJ, Kita H. Endogenous platelet-activating factor is critically involved in effector functions of eosinophils stimulated with IL-5 or IgG. *J Immunol* 1999; 162:2982–9.
- 71 Kato M, Abraham RT, Okada S, Kita H. Ligation of the beta 2 integrin triggers activation and degranulation of human eosinophils. *Am J Respir Cell Mol Biol* 1998; 18:675–86.
- 72 Kaneko M, Horie S, Kato M, Gleich GJ, Kita H. A crucial role for beta 2 integrin in the activation of eosinophils stimulated by IgG. *J Immunol* 1995; 155:2631–41.
- 73 Brown EJ, Bohnsack JF, Gresham HD. Mechanism of inhibition of immunoglobulin G-mediated phagocytosis by monoclonal antibodies that recognizes the Mac-1 antigen. *J Clin Invest* 1988; 81:365–75.
- 74 Abu-Ghazaleh RI, Fujisawa T, Mestecky J, Kyle RA, Gleich GJ. IgA-induced eosinophil degranulation. *J Immunol* 1989; 142:2393–400.
- 75 Lamkhieoued B, Gounni AS, Gruart V, Pierce A, Capron A, Capron M. Human eosinophils express a receptor for secretory component. Role in secretory IgA-dependent activation. *Eur J Immunol* 1995; 25:117–25.
- 76 Motegi Y, Kita H. Interaction with secretory component stimulates effector functions of human eosinophils but not of neutrophils. *J Immunol* 1998; 161:4340–6.
- 77 Kita H, Kaneko M, Bartemes KR et al. Does IgE bind to and activate eosinophils from patients with allergy? *J Immunol* 1999; 162:6901–11.
- 78 Bracke M, Dubois GR, Bolt K et al. Differential effects of the T helper cell type 2-derived cytokines IL-4 and IL-5 on ligand binding to IgG and IgA receptors expressed by human eosinophils. *J Immunol* 1997; 159:1459–65.
- 79 Zhu X, Hamann KJ, Munoz NM et al. Intracellular expression of Fc gamma RIII (CD16) and its mobilization by chemoattractants in human eosinophils. *J Immunol* 1998; 161:2574–9.
- 80 Tomassini M, Tsicopoulos A, Tai PC et al. Release of granule proteins by eosinophils from allergic and nonallergic patients with eosinophilia on immunoglobulin-dependent activation. *J Allergy Clin Immunol* 1991; 88:365–75.
- 81 Gounni AS, Lamkhieoued B, Ochiai K et al. High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 1994; 367:183–6.
- 82 Capron M, Soussi Gounni A, Morita M et al. Eosinophils: from low- to high-affinity immunoglobulin E receptors. *Allergy* 1995; 50:20–3.
- 83 Seminario MC, Saini SS, MacGlashan DW Jr, Bochner BS. Intracellular expression and release of Fc epsilon RI alpha by human eosinophils. *J Immunol* 1999; 162:6893–900.
- 84 Kuna P, Iyer M, Peerschke EI, Kaplan AP, Reid KB, Ghebrehiwet B. Human C1q induces eosinophil migration. *Clin Immunol Immunopathol* 1996; 81:48–54.
- 85 Gerard NP, Hodges MK, Drazen JM, Weller PF, Gerard C. Characterization of a receptor for C5a anaphylatoxin on human eosinophils. *J Biol Chem* 1989; 264:1760–6.
- 86 Zwirner J, Gotze O, Begemann G, Kapp A, Kirchhoff K, Werfel T. Evaluation of C3a receptor expression on human leucocytes by the use of novel monoclonal antibodies. *Immunology* 1999; 97:166–72.
- 87 Takafuji S, Tadokoro K, Ito K. Effects of interleukin (IL) -3 and IL-5 on human eosinophil degranulation induced by complement components C3a and C5a. *Allergy* 1996; 51:563–8.
- 88 Walsh GM, Kay AB. Binding of immunoglobulin classes to human neutrophils and eosinophils. *Clin Exp Immunol* 1986; 63:466–72.
- 89 Kim JT, Gleich GJ, Kita H. Roles of CD9 molecules in survival and activation of human eosinophils. *J Immunol* 1997; 159:926–33.
- 90 Egesten A, Malm J. Eosinophil leukocyte degranulation in response to serum-opsonized beads. C5a and platelet-activating factor (PAF) enhance ECP release, with roles for protein kinases A and C. *Allergy* 1998; 53:1–8.
- 91 Thorne KJ, Richardson BA, Mazza G, Butterworth AE. A new method for measuring eosinophil activating factors, based on the increased expression of CR3 alpha chain (CD11b) on the surface of activated eosinophils. *J Immunol Meth* 1990; 133:47–54.
- 92 Koenderman L, Kuijpers TW, Blom M, Tool AT, Roos D, Verhoeven AJ. Characteristics of CR3-mediated aggregation in human eosinophils: effect of priming by platelet-activating factor. *J Allergy Clin Immunol* 1991; 87:947–54.
- 93 Zhu X, Subbaraman R, Sano H et al. A surrogate method for assessment of beta (2)-integrin-dependent adhesion of human eosinophils to ICAM-1. *J Immunol Meth* 2000; 240:157–64.
- 94 Lindau M, Hartmann J, Sceppek S. Three distinct fusion processes during eosinophil degranulation. *Ann NY Acad Sci* 1994; 710:232–47.
- 95 Sceppek S, Lindau M. Focal exocytosis by eosinophils – compound exocytosis and cumulative fusion. *EMBO J* 1993; 12:1811–7.
- 96 Tai PC, Spry CJ. Studies on blood eosinophils. I. Patients with a transient eosinophilia. *Clin Exp Immunol* 1976; 24:415–22.
- 97 Tai PC, Spry CJ. The mechanisms which produce vacuolated and degranulated eosinophils. *Br J Haematol* 1981; 49:219–26.

- 98 Shukla A, Berglund L, Nielsen LP, Nielsen S, Hoffmann HJ, Dahl R. Regulated exocytosis in immune function: are SNARE-proteins involved? *Respir Med* 2000; 94:10–7.
- 99 Lacy P, Thompson N, Tian M et al. A survey of GTP-binding proteins and other potential key regulators of exocytotic secretion in eosinophils. Apparent absence of rab3 and vesicle fusion protein homologues. *J Cell Sci* 1995; 108:3547–56.
- 100 Young JD-E, Peterson CGB, Venge P, Cohn ZA. Mechanism of membrane damage mediated by human eosinophil cationic protein. *Nature* 1986; 321:613–6.
- 101 Abu-Ghazaleh RI, Gleich GJ, Prendergast FG. Interaction of eosinophil granule major basic protein with synthetic lipid bilayers: a mechanism for toxicity. *J Membr Biol* 1992; 128:153–64.
- 102 Gullberg U, Widegren B, Arnason U, Egesten A, Olsson I. The cytotoxic eosinophil cationic protein has ribonuclease activity. *Biochem Biophys Res Comm* 1986; 139:1239–42.
- 103 Rosenberg HF. Recombinant human eosinophil cationic protein. Ribonuclease activity is not essential for cytotoxicity. *J Biol Chem* 1995; 270:7876–81.
- 104 Domachowske JB, Dyer KD, Adams AG, Leto TL, Rosenberg HF. Eosinophil cationic protein/RNase 3 is another RNase A-family ribonuclease with direct antiviral activity. *Nucl Acids Res* 1998; 26:3358–63.
- 105 Domachowske JB, Dyer KD, Bonville CA, Rosenberg HF. Recombinant human eosinophil-derived neurotoxin/RNase 2 functions as an effective antiviral agent against respiratory syncytial virus. *J Infect Dis* 1998; 177:1458–64.
- 106 Yazdanbakhsh M, Tai PC, Spry CJ, Gleich GJ, Roos D. Synergism between eosinophil cationic protein and oxygen metabolites in killing of schistosomula of *Schistosoma mansoni*. *J Immunol* 1987; 138:3443–7.
- 107 Jong EC, Henderson WR, Klebanoff SJ. Bactericidal activity of eosinophil peroxidase. *J Immunol* 1980; 124:1378–82.
- 108 Mahmoud AF. Diseases due to helminths. In: Mandell, GL, Bennett, JE, Dolin, R, eds. *Principles and Practice of Infectious Diseases*, 5th edn. Churchill Livingstone.. Philadelphia, Penn., 2000: 2937–8.
- 109 Butterworth AE, Wassom DL, Gleich GJ, Loegering DA, David JR. Damage to *Schistosomula mansoni* induced directly by eosinophil major basic protein. *J Immunol* 1979; 122:221–9.
- 110 Hamann KJ, Gleich GJ, Checkel JL, Loegering DA, McCall JW, Barker RL. In vitro killing of microfilariae of *Brugia pahangi* and *Brugia malayi* by eosinophil granule proteins. *J Immunol* 1990; 144:3166–73.
- 111 Ackerman SJ, Gleich GJ, Loegering DA, Richardson BA, Butterworth AE. Comparative toxicity of purified human eosinophil granule cationic proteins for schistosomula of *Schistosoma mansoni*. *Am J Trop Med Hyg* 1985; 34:735–45.
- 112 Wassom DL, Gleich GJ. Damage to *Trichinella spiralis* newborn larvae by eosinophil major basic protein. *Am J Trop Med Hyg* 1979; 28:860–3.
- 113 Jong EC, Mahmoud AA, Klebanoff SJ. Peroxidase-mediated toxicity to schistosomula of *Schistosoma mansoni*. *J Immunol* 1981; 126:468–71.
- 114 Lehrer RI, Szklarek D, Barton A, Ganz T, Hamann KJ, Gleich GJ. Antibacterial properties of eosinophil major basic protein and eosinophil cationic protein. *J Immunol* 1989; 142:4428–34.
- 115 Klebanoff SJ, Agosti JM, Jorg A, Waltersdorff AM. Comparative toxicity of the horse eosinophil peroxidase-H₂O₂-halide system and granule basic proteins. *J Immunol* 1989; 143:239–44.
- 116 Kato M, Kephart GM, Talley NJ et al. Eosinophil infiltration and degranulation in normal human tissue. *Anat Rec* 1998; 252:418–25.
- 117 Schwarze J, Cieslewicz G, Hamelmann E et al. IL-5 and eosinophils are essential for the development of airway hyperresponsiveness following acute respiratory syncytial virus infection. *J Immunol* 1999; 162:2997–3004.
- 118 Jong EC, Klebanoff SJ. Eosinophil-mediated mammalian tumor cell cytotoxicity. role of the peroxidase system. *J Immunol* 1980; 124:1949–53.
- 119 Pretlow TP, Keith EF, Cryar AK, Bartolucci AA, Pitts AM, Pretlow TG 2d, Kimball PM, Boohaker EA. Eosinophil infiltration of human colonic carcinomas as a prognostic indicator. *Cancer Res* 1983; 43:2997–3000.
- 120 Fernandez-Acenero MJ, Galindo-Gallego M, Sanz J, Aljama A. Prognostic influence of tumor-associated eosinophilic infiltrate in colorectal carcinoma. *Cancer* 2000; 88:1544–8.
- 121 Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000; 105:651–63.
- 122 Hastie AT, Loegering DA, Gleich GJ, Kueppers F. The effect of purified human eosinophil major basic protein on mammalian ciliary activity. *Am Rev Respir Dis* 1987; 135:848–53.
- 123 Jacoby DB, Gleich GJ, Fryer AD. Human eosinophil major basic protein is an endogenous allosteric antagonist at the inhibitory muscarinic M2 receptor. *J Clin Invest* 1993; 91:1314–8.
- 124 Henderson WR, Chi EY, Klebanoff SJ. Eosinophil peroxidase-induced mast cell secretion. *J Exp Med* 1980; 152:265–79.
- 125 Weller PF, Bubley GJ. The idiopathic hypereosinophilic syndrome. *Blood* 1994; 83:2759–79.
- 126 Pincus SH, Ramesh KS, Wyler DJ. Eosinophils stimulate fibroblast DNA synthesis. *Blood* 1987; 70:572–4.
- 127 Wong DT, Weller PF, Galli SJ et al. Human eosinophils express transforming growth factor alpha. *J Exp Med* 1990; 172:673–81.
- 128 Wong DT, Elovic A, Matossian K et al. Eosinophils from patients with blood eosinophilia express transforming growth factor beta 1. *Blood* 1991; 78:2702–7.
- 129 Levi-Schaeffer F, Barkans J, Newman TM et al. Identification of interleukin-2 in human peripheral blood eosinophils. *Immunology* 1996; 87:155–61.
- 130 Kita H, Ohnishi T, Okubo Y, Weiler D, Abrams JS, Gleich GJ. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J Exp Med* 1991; 174:749.

- 131 Moqbel R, Ying S, Barkans J et al. Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. *J Immunol* 1995; 155:4939–47.
- 132 Dubucquoi S, Desreumaux P, Janin A et al. Interleukin 5 synthesis by eosinophils: association with granules and immunoglobulin-dependent secretion. *J Exp Med* 1994; 179:703.
- 133 Hamid Q, Barkans J, Meng Q et al. Human eosinophils synthesize and secrete interleukin-6 in vitro. *Blood* 1992; 80:1496–501.
- 134 Lacy P, Levi-Schaeffer F, Mahmudi-Azer S et al. Intracellular localization of interleukin-6 in eosinophils from atopic asthmatics and effects of interferon γ . *Blood* 1998; 91:2508–16.
- 135 Braun RK, Franchini M, Erard F et al. Human peripheral blood eosinophils produce and release interleukin-8 on stimulation with calcium ionophore. *Eur J Immunol* 1993; 23:956–60.
- 136 Nakajima H, Gleich GJ, Kita H. Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. *J Immunol* 1996, June 15; 156 (12):4859–66.
- 137 Nutku E, Gounni AS, Olivenstein R, Hamid Q. Evidence for expression of eosinophil-associated IL-12 messenger RNA and immunoreactivity in bronchial asthma. *J Allergy Clin Immunol* 2000; 106:288–92.
- 138 Lim KG, Wan HC, Bozza PT et al. Human eosinophils elaborate the lymphocyte chemoattractants: IL-16 (lymphocyte chemoattractant factor) and RANTES. *J Immunol* 1996; 156:2566.
- 139 Levi-Schaeffer F, Lacy P, Severs NJ et al. Association of granulocyte-macrophage colony-stimulating factor with the crystalloid granules of human eosinophils. *Blood* 1995; 85:2579–86.
- 140 Costa JJ, Matossian K, Resnick MB et al. Human eosinophils can express the cytokines tumor necrosis factor- α and macrophage inflammatory protein-1 α . *J Clin Invest* 1993; 91:2673–84.
- 141 Ohno I, Nitta Y, Yamauchi K et al. Eosinophils as a potential source of platelet-derived growth factor B-chain (PDGF-B) in nasal polyposis and bronchial asthma. *Am J Respir Cell Mol Biol* 1995; 13:639–47.
- 142 Horiuchi T, Weller PF. Expression of vascular endothelial growth factor by human eosinophils: upregulation by granulocyte macrophage colony-stimulating factor and interleukin-5. *Am J Respir Cell Mol Biol* 1997; 17:70–7.
- 143 Izumi S, Hirai K, Miyamasu M et al. Expression and regulation of monocyte chemoattractant protein-1 by human eosinophils. *Eur J Immunol* 1997; 27:816–24.
- 144 Ying S, Meng Q, Taborda-Barata L et al. Human eosinophils express messenger RNA encoding RANTES and store and release biologically active RANTES protein. *Eur J Immunol* 1996; 26:70–6.
- 145 Nakajima T, Yamada H, Iikura M et al. Intracellular localization and release of eotaxin from normal eosinophils. *FEBS Lett* 1998; 434:226–30.
- 146 Peters MS, Rodriguez M, Gleich GJ. Localization of human eosinophil granule major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin by immunoelectron microscopy. *Lab Invest* 1986; 54:656–62.
- 147 Holtsberg FW, Ozgur LE, Garsetti DE, Myers J, Egan RW, Clark MA. Presence in human eosinophils of a lysophospholipase similar to that found in the pancreas. *Biochem J* 1995; 309:141–4.
- 148 Blom M, Tool AT, Wever PC et al. Human eosinophils express, relative to other circulating leukocytes, large amounts of secretory 14-kD phospholipase A2. *Blood* 1998; 91:3037–43.
- 149 Calafat J, Janssen H, Tool A, Dentener MA, Knol EF, Egesten A. The bactericidal/permeability increasing protein (BPI) is present in specific granules of eosinophils. *Blood* 1998; 91:4770–5.
- 150 Hoijer MA, Melief MJ, Calafat J et al. Expression and intracellular localization of the human N-acetylmuramyl-L-alanine amidase, a bacterial cell wall-degrading enzyme. *Blood* 1997; 90:1246–54.
- 151 Mayeno AN, Hamann KJ, Gleich GJ. Granule-associated flavin adenine dinucleotide (FAD) is responsible for eosinophil autofluorescence. *J Leukoc Biol* 1992; 51:172–5.
- 152 Iozzo RV, MacDonald GH, Wight TN. Immunoelectron microscopic localization of catalase in human eosinophilic leukocytes. *J Histochem Cytochem* 1982; 30:697–701.
- 153 Mabilat-Pragnon C, Janin A, Michel L et al. Urokinase localization and activity in isolated eosinophils. *Thromb Res* 1997; 88:373–9.
- 154 Dunn WB, Spicer SS. Histochemical demonstration of sulfated mucosubstances and cationic proteins in human granulocytes and platelets. *J Histochem Cytochem* 1969; 17:668–74.
- 155 Rothenberg ME, Pomerantz JL, Owen Jr WF et al. Characterization of a human eosinophil proteoglycan, and augmentation of its biosynthesis and size by interleukin 3, interleukin 5, and granulocyte/macrophage colony stimulating factor. *J Biol Chem* 1988; 263:13901–8.
- 156 Johansson B, Malm J, Persson T, Janciauskiene S, Carlson J, Egesten A. Alpha-1-antitrypsin is present in the specific granules of human eosinophilic granulocytes. *Clin Exp Allergy* 2001; 31:379–87.