

## Chapter 8

# Mast cells, basophils and eosinophils

Three kinds of granulocyte—mast cells, basophils and eosinophils—are distinguished from neutrophils by the differential staining characteristics of their granules. In mast cells and basophils this is caused by the presence of an acidic proteoglycan; in eosinophils the characteristic granules contain several basic proteins. The basophil is a circulating cell whereas the mast cell is sessile and present throughout the body but chiefly in perivascular connective tissue, epithelia and lymph nodes. There is heterogeneity within the mast cell population and dye binding is considerably affected by the method of fixation as well as the individual stains used. In appropriately fixed sections, the granules of mucosal and connective tissue mast cells differ in their staining properties. Mucosal mast cells have some features in common with basophils (which contrast with connective tissue mast cells), i.e. they are smaller, short lived, have chondroitin sulphate as acidic proteoglycan, are resistant to the inhibitory effect of sodium cromoglycate and require T cells for their growth and differentiation. Basophils have been identified in some forms of T cell mediated immune responses, e.g. Jones–Mote or cutaneous basophil hypersensitivity (see Chapter 14), and the vagaries of fixation and staining techniques have probably caused them to be overlooked in other situations.

Mast cell **degranulation** has a general role in immunity by regulating the egress of inflammatory cells and molecules through endothelial tight

junctions whenever a local inflammatory response is required to deal with a focus of infection (see Fig. 1.6 and Table 8.1). This increase in capillary permeability may be partly a result of the contraction of endothelial cells similar to the effect on smooth muscle fibres elsewhere. It is likely that the remarkable variation in permeability that occurs in the postcapillary venule of the lymph node is regulated by a similar process of IgE or complement-mediated mast cell degranulation as mast cells are found plentifully at the corticomedullary junction of lymph nodes, when appropriate fixation and staining methods are used. There is evidence that, in particular, mast cell derived tumour necrosis factor is important in promoting the lymph node enlargement that accompanies an immune response. By contrast, the inappropriate activation of mast cells is one of the principal causes of allergic inflammation (see Chapters 13 and 14).

### Triggering of mast cells and basophils

Mast cells and basophils possess surface **Fc receptors** with a high affinity for IgE (FcεRI). Mast cells become activated either when surface-bound IgE molecules become cross-linked by antigen (or experimentally by anti-IgE) or following the local release of the **anaphylatoxins** C3a or C5a for which mast cells also bear receptors. In either case, a complex series of events follows in which various

**Chapter 8** Mast cells, basophils and eosinophils

membrane enzymes are activated, calcium ions enter the cell, and granules and their preformed mediator contents are released by exocytosis (Fig. 8.1). New mediators generated from arachidonic acid metabolism are released over a longer time-scale, and were traditionally referred to as **slow reacting substance** of anaphylaxis (SRS).

The initial step involves the activation of a serine esterase followed by the activation of methyl transferases acting on membrane phospholipids, on the one hand, and adenylyl cyclase which generates an increase in intracellular cyclic adenosine monophosphate (cAMP) and protein kinase activity, on the other. Phospholipid methylation and the action of phospholipases also lead to protein kinase activation (through the generation of diacyl glycerol) and are associated with three other important events:

- 1** the opening of membrane **calcium channels** and the release of **intracellular calcium** (the latter occurring via the generation of inositol triphosphate);

- 2** the generation of **fusagenic lipids** which encourage the fusion of perigranular and cell surface membranes; and

- 3** the production of a supply of **arachidonic acid** from which various newly synthesized mediators are derived.

The activation of adenylyl cyclase is critical for mediator release although its inhibition does not prevent phospholipid methylation. Once calcium enters the cell it is bound by calmodulin, which increases the activity of various enzymes (including protein kinases) and promotes the processes by which cytoskeletal proteins cause the contraction of microfilaments, leading to the extrusion of the granules and their contents. The antiallergic drug **sodium cromoglycate** blocks mast cell degranulation and is thought to act by preventing the transmembrane influx of calcium ions.

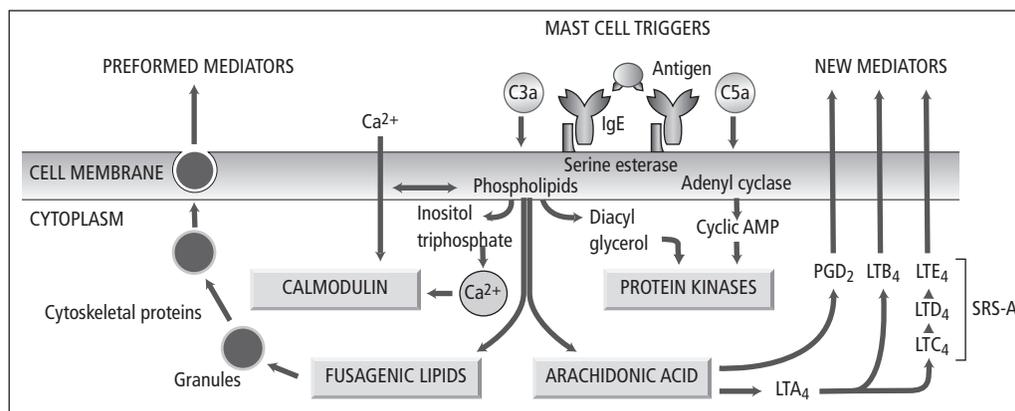
**Mast cell mediators**

**Preformed mediators**

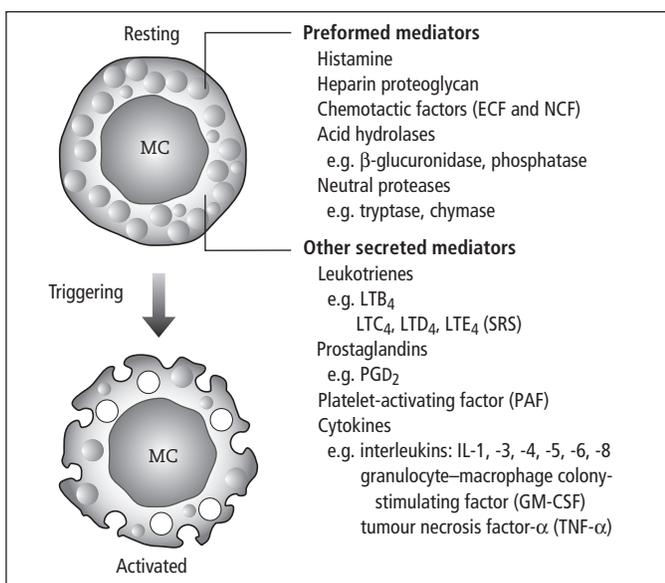
The preformed mediators present within mast cell granules consist of **histamine**, eosinophil and neutrophil **chemotactic factors** (ECF and NCF), heparin proteoglycan, acid hydrolases (e.g. aryl sulphatase and  $\beta$ -glucuronidase) and neutral proteases (e.g. tryptase and chymase; Fig. 8.2). Histamine is a small molecule that contracts smooth

**Table 8.1** Inflammatory effects of mediators released by mast cells.

Vasodilatation
Vascular permeability
Smooth muscle contraction
Leucocyte chemotaxis



**Figure 8.1** Processes involved in mast cell triggering and mediator release. See text for further details. LT, leukotriene; PG, prostaglandin.



**Figure 8.2** Stored and secreted products of the mast cell (MC).

muscle and increases vascular permeability. It is present in mast cell granules as part of a protein complex containing the proteoglycan **heparin**. Heparin is replaced by **chondroitin sulphate** in the basophil. Heparin is anticoagulant and anti-complementary and may have a role in promoting the diffusion of mast cell mediators following degranulation despite activation of the coagulation pathway, as well as contributing to the packaging and stabilization of mediators within the granules. The chemotactic factors released by mast cells not only attract other granulocytes, but also increase the expression of their C3 receptors and have a stimulatory effect on the respiratory burst and the generation of oxygen-derived products.

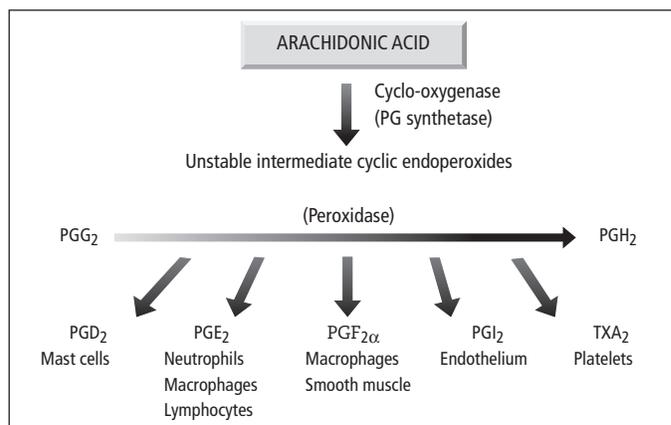
### Secondary mediators

SRS release takes place at a slower tempo than histamine release, the latter being a preformed mediator whereas the former has to be freshly synthesized. These 'secondary' mediators are lipid derivatives of **arachidonic acid** formed via two different pathways of metabolism under the control of cyclo-oxygenase and lipoxygenase enzymes (Figs 8.3 and 8.4). They include **SRS** (now known to consist of a combination of three different

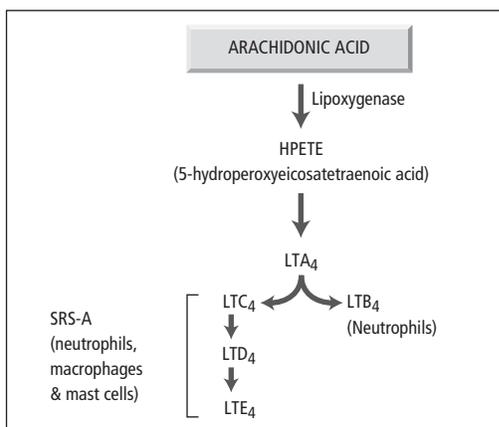
**leukotrienes**:  $LTC_4$ ,  $LTD_4$  and  $LTE_4$ );  $LTB_4$  (a potent chemotactic agent); the prostaglandins  $PGE_2$ ,  $PGD_2$  and  $PGF_{2\alpha}$ ; and platelet-activating factor (PAF). The microsomal enzyme **cyclo-oxygenase** (also called prostaglandin synthetase) converts arachidonic acid to unstable intermediate cyclic endoperoxides ( $PGG_2$  and  $PGH_2$ ) which are then further metabolized to form stable prostaglandin mediators specific for each cell type. The predominant prostaglandin formed in mast cells is  $PGD_2$ , which causes vasodilatation, contracts smooth muscle and is chemotactic for neutrophils.  $PGE_2$  is produced in neutrophils, macrophages and lymphocytes and is a potent vasodilator. Macrophages also synthesize  $PGF_{2\alpha}$  which contracts smooth muscle. Other stable prostaglandins produced by this pathway include thromboxane ( $TXA_2$ ), formed in platelets, and prostacyclin ( $PGI_2$ ), which is produced by endothelial cells.

The leukotrienes are derived from the metabolic oxidation of arachidonic acid by the **lipoxygenase** pathway in which the unstable 5-hydroperoxyeicosatetraenoic acid (HPETE) is converted initially to the leukotriene  $LTA_4$ , which, depending on the cell concerned, either metabolizes to  $LTB_4$  (e.g. in neutrophils) or is converted by the SRS pathway to  $LTC_4$ ,  $LTD_4$  and  $LTE_4$  (Fig. 8.4). This

**Chapter 8** Mast cells, basophils and eosinophils



**Figure 8.3** Products of the cyclo-oxygenase pathway and the cell types in which they are formed. PG, prostaglandin; TXA<sub>2</sub>, thromboxane.



**Figure 8.4** Products of the lipoxygenase pathway and the cell types in which they are formed.

themselves may have more of an immunological role than previously thought. They have Fc receptors for both IgG and IgE. Activation via the IgG receptor causes release of 5HT whereas triggering by IgE generates oxygen metabolites which have a lytic effect on some parasites, e.g. schistosomes.

**Cytokines**

Activated mast cells secrete a number of proinflammatory cytokines, including tumour necrosis factor- $\alpha$  and chemokines (e.g. interleukin-8). They also produce IL-4, which promotes the switching of B cells to IgE production as well as Th2 cell development, and IL-5 which promotes the differentiation and activation of eosinophils.

**Eosinophils**

Eosinophils are distinguished by the striking affinity of their granules for acid or aniline dyes. They form a small proportion of peripheral blood leucocytes (1–5%) but are more prevalent in tissues. They probably share a common precursor with the basophil and show a later differentiation stage in the blood comparable to macrophage activation. They become more plentiful (in blood and relevant tissues) in allergic and parasitic diseases and their functions can be divided into effects on parasites and the inflammatory process.

Various factors have been identified that promote eosinophil proliferation and differentiation,

latter process occurs in various granulocytes and mononuclear cells and it is possible that at least two cell types are required for the full expression of leukotriene synthesis. SRS is a particularly potent constrictor of smooth muscle and a vasodilator; it also causes mucus secretion. PGD<sub>2</sub> is the major arachidonic acid metabolite formed in connective tissue mast cells whereas LTC<sub>4</sub> production is prominent in mucosal mast cells and basophils.

**Platelet-activating factors** are phospholipids that cause calcium-dependent release of histamine and 5-hydroxytryptamine (5HT) from platelets and are also able to degranulate neutrophils and contract smooth muscle. Platelets

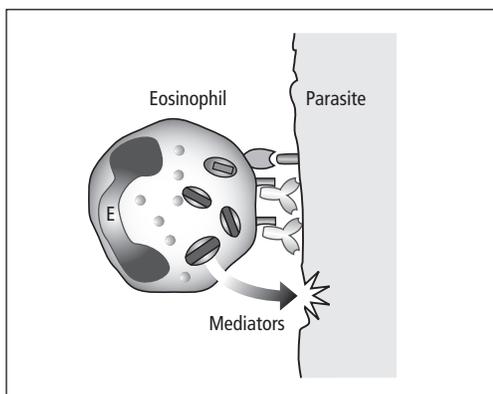
e.g. granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-3 and IL-5 and other eosinopoietic factors. Eosinophils also show a brisk chemotactic response to several materials liberated during the immune response, e.g. **ECF** (from mast cells), C5a and certain chemokines. ECF and C5a display synergism in their chemotactic effects on eosinophils.

Eosinophils phagocytose poorly but degranulate promptly in the presence of chemotactic factors and when membrane-bound IgG or IgE is cross-linked by antigen, i.e. exocytosis is more marked than endocytosis following triggering of their surface membrane, in contrast to the neutrophil. Eosinophils have Fc receptors for both IgG and IgE isotypes (see Table 7.2): they express low-affinity receptors for IgE (Fc<sub>ε</sub>RII), as well as high-affinity receptors (Fc<sub>ε</sub>RI) when activated. Like neutrophils, they also possess C3b receptors. They are able to form phagolysosomes following membrane triggering but this phenomenon is much less marked than in the neutrophil, and eosinophils display only limited proteolytic activity. A prominent role of neutrophils is the intracellular digestion of microbes (e.g. bacteria) which are readily phagocytosed. Eosinophils are more effective in the extracellular digestion of infectious agents that are too large to be engulfed (e.g. parasitic worms such

as schistosomes and helminths) (Fig. 8.5). Some of the contrasting features of mast cells, eosinophils and neutrophils are summarized in Table 8.2.

**Eosinophil products** (Fig. 8.6)

Eosinophils display an oxidative burst with generation of H<sub>2</sub>O<sub>2</sub> and, probably, superoxide, but it is uncertain whether they produce the other more



**Figure 8.5** Extracellular digestion by an eosinophil. The eosinophil is targeted to the surface of a parasitic worm opsonized by IgG or IgE antibodies and C3b. Lytic mediators are exocytosed onto the parasite surface (see text and Fig. 8.6).

**Table 8.2** Contrasting features of connective tissue mast cells, eosinophils and neutrophils.

	<b>Mast cells</b>	<b>Eosinophils</b>	<b>Neutrophils</b>
Lifespan	Long lived	Long lived	Short lived
Dynamics	Sessile	Mobile	Mobile
Chemotactic response	–	+++ (ECF, C5a, chemokines)	+++ (NCF, C5a, chemokines)
Degranulation response (exocytosis)	+++	+++	+
Phagocytosis (endocytosis)	–	+	+++
Lytic ability	–	+++ (basic proteins, O radicals)	+++ (lysosomal enzymes, O radicals)
Receptors for cell triggering	IgE C3a C5a	IgE IgG C3b	IgG IgA C3b
Biological role	Gatekeeper, proinflammatory	Antihelminth, pro- or anti-inflammatory	Antibacterial

Chapter 8 Mast cells, basophils and eosinophils

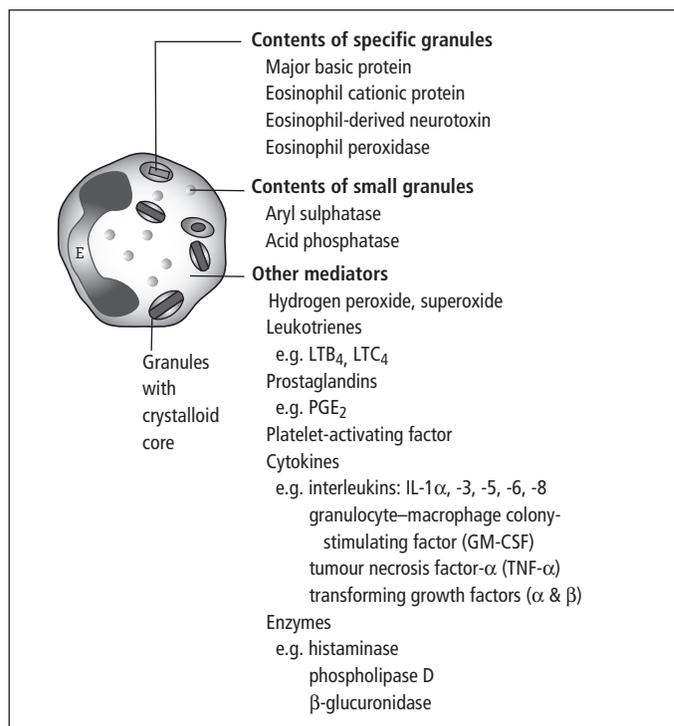


Figure 8.6 Stored and secreted products of the eosinophil.

lytic oxygen radicals found in the neutrophil (see Fig. 7.4). Eosinophil peroxidase (EPO) is different from myeloperoxidase (MPO) but may be able to work in concert with hydrogen peroxide and iodide or chloride ions to lyse some microorganisms, e.g. *Trichinella*. However, the major source of lytic activity in the eosinophil is the basic or **cationic proteins** contained within characteristic granules which are freely exocytosed during the degranulation response and are directly toxic to parasites, e.g. schistosomes, as well as to host cells.

The characteristic granules have a crystalloid core consisting largely of a **major basic protein** and a peripheral matrix containing other basic proteins, e.g. eosinophil cationic protein, and eosinophil-derived neurotoxin, as well as eosinophil peroxidase. Separate smaller granules contain aryl sulphatase and acid phosphatase. Eosinophil granules do not contain lysozyme. The exact location of other enzymes released by the cell, e.g. histaminase,  $\beta$ -glucuronidase and phos-

pholipase D, is unclear. The protein, which forms Charcot-Leyden crystals in various tissues and body fluids subjected to eosinophil degranulation, is a lysophospholipase that resides in the plasma membrane of eosinophils (and basophils). Eosinophils also metabolize arachidonic acid to produce large amounts of PAF leukotrienes, e.g.  $LTB_4$  and  $LTC_4$ , and  $PGE_2$ . The cationic proteins and arachidonic acid metabolites derived from eosinophils contribute, together with mast cell products, to the acute and chronic phases of allergic inflammation. However, several of the other eosinophil products have an inhibitory effect on mast cell mediators (Table 8.3), and so may be anti-inflammatory.

Eosinophils also produce a number of cytokines (Fig. 8.6). Some of these act as autocrine growth factors (i.e. GM-CSF, IL-3 and IL-5), whereas others may have proinflammatory activity (e.g. IL-1, IL-6, IL-8, TNF- $\alpha$ ) or anti-inflammatory effects (e.g. TGF- $\beta$ ).

**Table 8.3** Interactions between mast cell and eosinophil products.

Inflammatory mediators produced by mast cells	Inhibitory factors produced by eosinophils
Histamine	Histaminase PGE <sub>2</sub>
Heparin	Major basic protein
SRS	Aryl sulphatase
LTB	Peroxidase
PGD <sub>2</sub>	
PAF	Phospholipase

### Hyper-eosinophilic syndrome

The release of inflammatory mediators can have serious complications in patients with the **hyper-eosinophilic syndrome**. These consist of endomyocardial fibrosis and thromboembolic disease, largely associated with the liberation of toxic basic proteins and PAF, respectively.

#### Key points

- 1 Mast cells and basophils are activated by multivalent antigens cross-linking surface-bound IgE molecules, or by anaphylatoxins (C3a and C5a); this induces the release of inflammatory mediators.
- 2 Some mast cell mediators are preformed and stored in granules (e.g. histamine) and are exocytosed immediately upon activation. Other mediators are synthesized *de novo* (e.g. leukotrienes and prostaglandins). Mast cells also secrete cytokines.
- 3 Eosinophils possess granules containing lytic mediators (e.g. major basic protein), which are exocytosed upon interaction with IgE or IgG molecules bound to the surface of, for example, parasitic worms.
- 4 Eosinophils also secrete some mediators, that promote inflammation (e.g. arachidonic acid metabolites and cytokines), whereas others have anti-inflammatory effects on mast cell mediators.