

Eosinophils: Biological Properties and Role in Health and Disease

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SUMMARY

Eosinophils are pleiotropic multifunctional leukocytes involved in initiation and propagation of diverse inflammatory responses, as well as modulators of innate and adaptive immunity. In this review, the biology of eosinophils is summarized, focusing on transcriptional regulation of eosinophil differentiation, characterization of the growing properties of eosinophil granule proteins, surface proteins and pleiotropic mediators, and molecular mechanisms of eosinophil degranulation. New views on the role of eosinophils in homeostatic function are examined, including developmental biology and innate and adaptive immunity (as well as their interaction with mast cells and T cells) and their proposed role in disease processes including infections, asthma, and gastrointestinal disorders. Finally, strategies for targeted therapeutic intervention in eosinophil-mediated mucosal diseases are conceptualized.

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INTRODUCTION

Eosinophils are multifunctional leukocytes implicated in the pathogenesis of numerous inflammatory processes including parasitic helminth, bacterial and viral infections, tissue injury, tumor immunity, and allergic diseases (Gleich & Loeffering 1984; Weller 1994; Rothenberg 1998). In response to the diverse stimuli, eosinophils are recruited from the circulation into inflammatory foci where they modulate immune responses through an array of mechanisms. Triggering of eosinophils by engagement of receptors for cytokines, immunoglobulins, and complement can lead to the secretion of an array of proinflammatory cytokines, such as interleukin (IL)-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-16, IL-18, and transforming growth factor (TGF)- α/β , chemokines such as CCL5/RANTES and CCL11/eotaxin-1, and lipid mediators such as platelet-activating factor (PAF) and leukotriene (LT) C_4 (Kita 1996). These molecules have proinflammatory effects that include upregulation of adhesion systems, modulation of cellular trafficking and activation and regulation of vascular permeability, mucus secretion, and smooth muscle constriction. Eosino-

phils can initiate antigen-specific immune responses by acting as antigen-presenting cells. Furthermore, eosinophils can serve as major effector cells inducing tissue damage and dysfunction by releasing toxic granule proteins and lipid mediators (Gleich & Adolphson 1986).

In this chapter, we summarize eosinophil surface marker expression and the growing number of properties defined for eosinophil degranulation. We review the molecular mechanisms involved in eosinophil development and trafficking, including the role of the transcription factors GATA-1, PU.1, and c/EBP members and the eosinophil selective cytokine IL-5 and the eotaxin subfamily of chemokines. Furthermore, we discuss the views on the role of eosinophils in homeostatic function, including developmental biology and innate and adaptive immunity, and in disease processes including infections, asthma, and gastrointestinal disorders.

EOSINOPHIL GRANULE PROTEINS

Eosinophils contain up to four different populations of secretory organelles: crystalloid granules, primary granules, small granules, and secretory vesicles. The largest of

the secretory organelles are the crystalloid granules (0.5–0.8 µm in diameter), which store the majority of granule proteins in eosinophils. The unique crystalloid granules are so called because they contain an intensely staining electron-dense crystalline core surrounded by an electron-lucent matrix when cells are stained and imaged by electron microscopy. Most of the granule proteins packaged into the crystalloid granules are composed of four highly basic proteins. Major basic protein (MBP) is crystallized in the core of the crystalloid granule, where it accounts for virtually all the protein (Gleich *et al.* 1973; Lewis *et al.* 1978). Eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN) reside in the granule matrix (Egsten *et al.* 1986; Peters *et al.* 1986). The primary granules appear during the promyelocytic stage of eosinophil development and are enriched in Charcot–Leyden crystal (CLC) protein. Small secretory vesicles have also been identified that overlap in their contents with those of small granules, and are packed densely in the cytoplasm of eosinophils. The biology of the major cationic proteins in eosinophils has been reviewed in detail (Gleich & Adolphson 1986; Walsh 2001); their functions are summarized in Table 12.1.

MAJOR BASIC PROTEIN

As one of the most highly cationic proteins synthesized by eosinophils, MBP is expressed as two different homologs (MBP1 and MBP2). MBP is a small protein that consists of single polypeptide chain of 117 amino acids, with a molecular mass of 13.8 kDa and a high isoelectric point (> 11), which cannot be measured accurately due to its extremely basic nature (Hamann *et al.* 1991). Its basicity is due to the presence of 17 arginine residues, and it also contains nine cysteine residues that enable it to form disulfide bonds. The cDNA for MBP encodes a pre-prosequence that includes a putative signal peptide and an acidic 90-amino acid prosequence that may serve to neutralize MBP's highly basic charge as it is processed through the Golgi and transported to the granule, where the prosequence is cleaved (Barker *et al.* 1988; McGrogan *et al.* 1988; Popken-Harris *et al.* 1998). MBP is among the most abundant proteins in eosinophils, with as much as 250 pg/cell detected in guinea-pig eosinophils, while comparatively less is found in human eosinophils (5–10 pg/cell). MBP1 can also be detected in basophil granules, although there is considerably less expressed than in eosinophils (Ackerman *et al.* 1983). Mature eosinophils lose the ability to transcribe mRNA

Table 12.1 Functions of eosinophil cationic granule proteins.

MBP

- Toxicity toward helminthic parasites such as schistosomulae of *Schistosoma mansoni* (Butterworth 1984; Ackerman *et al.* 1985; Gleich & Adolphson 1986)
 - Cytotoxicity toward airway epithelium (Frigas *et al.* 1980; Hastie *et al.* 1987; Hisamatsu *et al.* 1990; Furuta *et al.* 2005)
 - Bronchoconstriction and hyperresponsiveness on aerosolization in rats and monkeys (Gundel *et al.* 1991; Coyle *et al.* 1993; Uchida *et al.* 1993)
 - Platelet agonist (Rohrbach *et al.* 1990)
 - Activation of complement via classical and alternative pathways (Weiler *et al.* 1992, 1995)
 - Antibacterial properties (Lehrer *et al.* 1989)
 - Activation of remodeling factors from epithelial cells (Pégrier *et al.* 2006)
 - Increased cutaneous vasopermeability (Davis *et al.* 2003)
 - Stimulation of signaling pathways and mediator release from mast cells, neutrophils and basophils (Zheutlin *et al.* 1984; Haskell *et al.* 1995; Page *et al.* 1999; Shenoy *et al.* 2003)
- ### ECP
- Ribonuclease activity (100 times less potent than EDN) (Slifman *et al.* 1986)
 - Toxicity toward helminthic parasites and mammalian epithelial cells (McLaren *et al.* 1981; Ackerman *et al.* 1985)
 - Bactericidal properties (Lehrer *et al.* 1989)
 - Induction of Gordon phenomenon (Durack *et al.* 1979; Fredens *et al.* 1982)
 - Promotion of mast cell degranulation (Zheutlin *et al.* 1984)
- ### EDN
- Weakly toxic for parasites and mammalian cells (Ackerman *et al.* 1985)
 - Induction of Gordon phenomenon (Durack *et al.* 1979)
 - Antiviral activity in respiratory infection (Rosenberg & Domachowske 2001)
- ### EPO
- Toxic for mammalian cells and degradative toward connective tissue via ability to form hypohalous acids (Slungaard & Mahoney 1991; Wang & Slungaard 2006)
 - Cytotoxicity toward airway epithelium (Brottman *et al.* 1996; Pégrier *et al.* 2006)
 - Bactericidal, membrane lysis, and signaling pathway effects (Wang & Slungaard 2006)
 - Induction of oxidative damage and mutagenesis of DNA and RNA (Shen *et al.* 2000; Henderson *et al.* 2001)

encoding MBP, indicating that all the MBP stored in crystalloid granules is synthesized during early eosinophil development prior to maturation (Popken-Harris *et al.* 1998).

Plasma concentrations of MBP are elevated in the sera of pregnant women, with a peak 2–3 weeks before

parturition. Placental eosinophils are few in numbers, and MBP1 has been shown to be synthesized by placental $\times$ cells and placental site giant cells (Maddox *et al.* 1984). MBP2 is exclusively expressed by eosinophils, and may be a more specific marker for elevated eosinophils in patients with eosinophilia than MBP1 (Plager *et al.* 2006). The classical role of eosinophils in protection against parasitic infections has been supported by the toxicity of MBP against helminthic worms (O'Donnell *et al.* 1983; Butterworth 1984; Ackerman *et al.* 1985; Gleich & Adolphson 1986). MBP has also been shown to be cytotoxic to airways and may be at least partly responsible for tissue damage associated with eosinophil infiltration in bronchial mucosa in asthma (Frigas *et al.* 1980; Hisamatsu *et al.* 1990; Furuta *et al.* 2005). The toxic effect of MBP is thought to result from increased membrane permeability through surface charge interactions leading to perturbation of the cell-surface lipid bilayer (Wasmoen *et al.* 1988).

EOSINOPHIL CATIONIC PROTEIN

ECP is a member of a subfamily of ribonuclease (RNase) A multigenes expressed in eosinophils, with approximately 15–25 pg synthesized per cell in human eosinophils. Similarly to MBP, ECP is a single-chain cationic polypeptide with a pI > 11. On molecular sizing, ECP displays marked heterogeneity, as a result of differential glycosylation, with a molecular mass ranging between 16 and 21.4 kDa. Two isoforms, ECP-1 and ECP-2, have been identified using heparin Sepharose chromatography (Gleich *et al.* 1986). The cDNA for ECP encodes a leader sequence of 27 amino acids and a mature protein of 133 amino acids with a calculated molecular mass of 15.6 kDa (Barker *et al.* 1989; Rosenberg *et al.* 1989). The amino acid sequence is 66% homologous to EDN and 31% homologous to human pancreatic ribonuclease. ECP does have ribonuclease activity but is 100 times less potent than EDN (Slifman *et al.* 1986). ECP is bactericidal, promotes degranulation from mast cells, and is toxic to helminthic parasites on its own (Gleich *et al.* 1986; Lehrer *et al.* 1989). The mechanism of action of ECP is thought to involve pore formation in target membranes, which is apparently not dependent on its RNase activity (Young *et al.* 1986). ECP was originally characterized for its ability to elicit the Gordon phenomenon (neurotoxicity causing stiffness, ataxia and paralysis) when injected into the cranial ventricles of rabbits (Durack *et al.* 1979).

EOSINOPHIL-DERIVED NEUROTOXIN

A second member of the RNase A multigene family, EDN, also called EPX (Slifman *et al.* 1986), is expressed in eosinophils and is less basic than ECP or MBP, with a pI of 8.9 due to a smaller number of arginine residues in its sequence. EDN is a single-chain polypeptide with an observed molecular mass of 18.6 kDa. EDN expression is not restricted to eosinophils, as it is also detected in mononuclear cells and possibly neutrophils. ECP and EDN share high sequence homology of 70% at the amino acid level for the pre-form of both proteins, suggesting that these proteins derived from the same gene during evolutionary development (Hamann *et al.* 1990). Eosinophils express approximately 10 pg of EDN per cell, with marked variation between individuals. EDN similarly induces the Gordon phenomenon when injected intracranially in laboratory animals (Durack *et al.* 1979). EDN is also implicated in antiviral activity against respiratory infections (Rosenberg & Domachowske 2001). The gene family expressing ECP and EDN has one of the highest rates of mutation in the primate genome, ranking with those of immunoglobulins, T-cell receptors, and major histocompatibility complex (MHC) classes (Rosenberg *et al.* 1995). This extreme rate of mutation suggests that evolutionary constraints acting on the ECP/EDN subfamily have promoted the acquisition of a specialized antiviral activity, inferred by the high mutation rates of other genes commonly associated with host protection against viral infection.

EOSINOPHIL PEROXIDASE

EPO is a heme-containing haloperoxidase with a high pI (> 11) composed of two subunits: a heavy chain of 50–57 kDa and a light chain of 11–15 kDa. EPO has 68% sequence identity to neutrophil myeloperoxidase, suggesting that a peroxidase multigene family may have developed through gene duplication (Ten *et al.* 1989; Hamann *et al.* 1991). Eosinophils store approximately 15 pg/cell of EPO. The functional role of EPO is associated with bacterial killing. EPO catalyzes the peroxidative oxidation of halides (such as bromide, chloride, and iodide) and pseudohalides (thiocyanate) present in the plasma together with hydrogen peroxide generated by dismutation of superoxide produced during respiratory burst (Weiss *et al.* 1986; Mayeno *et al.* 1989; Thomas *et al.* 1995). This reaction leads to the formation of bactericidal hypohalous acids, particularly hypobromous acid, under physiologic conditions. Eosinophils are

robust producers of extracellular superoxide due to expression of high levels of the enzyme complex that generates superoxide (NADPH oxidase) (Someya *et al.* 1997) and preferential assembly of the enzyme complex at the cell surface (Lacy *et al.* 2003).

CYTOKINES

Eosinophils can synthesize and secrete at least 35 important inflammatory and regulatory cytokines, chemokines, and growth factors (Table 12.2). Many of these cytokines are potent inducers of immune responses in asthma, eczema, rhinitis, and other inflammatory diseases. Those eosinophil-derived cytokines that have been quantified generally appear to be generated in relatively small amounts, suggesting an autocrine, paracrine, or juxtacrine role in regulating the function of the micro-environment. However, in some circumstances, eosinophils are the chief producers of cytokines such as TGF- β , which is linked with tissue remodeling in a variety of eosinophil-associated diseases, such as asthma (Kay *et al.* 2004). A major distinction in cytokine production between eosinophils and T cells, which generate much larger quantities of cytokines, is that eosinophils store their cytokines intracellularly as preformed mediators. Several eosinophil cytokines have been shown to be stored in crystalloid granules and small secretory vesicles, and possess bioactivity on their release (Lacy & Moqbel 2000). This allows the immediate release of cytokines on eosinophil activation, instead of the several hours or days required to generate cytokines from T cells. For example, release of the chemokine RANTES was shown to occur within 60–120 min of eosinophil stimulation by interferon (IFN)- γ . This was related to rapid mobilization (within 10 min) of RANTES in small secretory vesicles that translocated this chemokine to the cell membrane prior to its release (Fig. 12.1). Cytokines generated by eosinophils are discussed in more detail in several comprehensive reviews (Lacy & Moqbel 1997, 2000; Moqbel & Lacy 1998).

OTHER EOSINOPHIL-DERIVED MEDIATORS

A major constituent of the human eosinophil is CLC protein, also known as galectin-10 (Ackerman *et al.* 2002). CLC is a hydrophobic protein of molecular mass 17.4 kDa that was thought to possess a weak lysophospholipase activity, but instead modulates this by interacting with eosinophil lysophospholipases. It is synthesized at very high levels by eosinophils, and is

produced at lesser levels in basophils. CLC possesses strong sequence homology to the carbohydrate-binding galectin family of proteins, hence its designation as galectin-10. CLC was first characterized by Charcot and Robin in 1853 for its abundance in sputum and fecal samples from patients with severe respiratory and gastrointestinal eosinophilia. Its release results in the formation of distinct, needle-shaped structures that are colorless, measuring 20–40 μ m in length and 2–4 μ m across. However, the function of CLC remains obscure.

In addition, the eosinophil contains a number of other granule-stored enzymes whose exact role in eosinophil function has not been defined (Spry 1988). They include acid phosphatase (large amounts of which have been isolated from eosinophils), collagenase, arylsulfatase B, histaminase, phospholipase D, catalase, nonspecific esterases, and vitamin B₁₂-binding protein. Eosinophils are also a source of matrix metalloproteases, which have an important role in cell transmigration and inflammation (Ohno *et al.* 1997; Okada *et al.* 1997; Schwingshackl *et al.* 1999; Gauthier *et al.* 2003; Wiehler *et al.* 2004), although much less is produced than from monocytes, macrophages, and neutrophils. The intracellular location of matrix metalloprotease-9 has been localized to perinuclear regions and not the crystalloid granules (Ohno *et al.* 1997).

EOSINOPHILS EXPRESS SNARE ISOFORMS

Secretory cells from diverse biological systems express components of a fusion complex of membrane-bound proteins known as the SNARE (SNAP Receptor) complex, which is essential for vesicular docking and fusion (Sollner *et al.* 1993; Sutton *et al.* 1998). This complex, originally characterized in neuronal cells, is composed of VAMP-1 (vesicle-associated membrane protein, also known as synaptobrevin-1), syntaxin-1, and SNAP-25 (synaptosome-associated protein of 25 kDa). These molecules are categorized into two groups, namely vesicular SNAREs (v-SNAREs), which bind to plasma membrane target SNAREs (t-SNAREs). The SNARE molecules form a coiled-coil structure with four separate α -helices contributed by three different molecules during vesicle docking with the plasma membrane. The binding region associated with the four α -helices is known as the SNARE motif. Fusion of the granule membrane with the plasma membrane is dependent on cytosolic NSF (*N*-ethylmaleimide-sensitive factor) and α , β , or γ -SNAP (soluble NSF-attachment protein)-mediated disassembly of the SNARE complex (Sollner *et al.* 1993). Cleavage of

Table 12.2 Cytokine generation by eosinophils.

Cytokine	Products	Stored protein in resting cells (/10 ⁶ cells)	Intracellular site of storage
<i>Interleukins</i>			
Interleukin-1 α	mRNA, protein	–	–
Interleukin-2	mRNA, protein	6 $\pm$ 2 pg	Crystalloid granules (core)
Interleukin-3	mRNA, protein	–	–
Interleukin-4	mRNA, protein	$\sim$ 75 $\pm$ 20 pg	Crystalloid granules (core)
Interleukin-5	mRNA, protein	–	Crystalloid granules (core/matrix?)
Interleukin-6	mRNA, protein	25 $\pm$ 6 pg	Crystalloid granules (matrix)
Interleukin-9	mRNA, protein	–	–
Interleukin-10	mRNA, protein	– 25 pg	–
Interleukin-11	mRNA	–	–
Interleukin-12	mRNA, protein	–	–
Interleukin-13	mRNA, protein	–	–
Interleukin-16	mRNA, protein	1.6 $\pm$ 0.8 ng	–
Interleukin-17			
Leukemia inhibitory factor	mRNA, protein	–	–
<i>Interferons and others</i>			
Interferon (IFN)- γ	mRNA, protein	–	–
Tumor necrosis factor α	mRNA, protein	–	Crystalloid granules (matrix)
Granulocyte-macrophage colony-stimulating factor	mRNA, protein	15.1 $\pm$ 0.3 pg	Crystalloid granules (core)
<i>Chemokines</i>			
Epithelial cell-derived neutrophil activating peptide (ENA-78/CXCL5)	mRNA, protein	12 $\pm$ 2 pg	–
Eotaxin (CCL11)	mRNA, protein	19 $\pm$ 4 pg	Crystalloid granules
Growth-related oncogene (GRO α /CXCL1)	mRNA, protein	–	–
Interleukin-8 (CXCL8)	mRNA, protein	140 pg	Cytoplasmic
IFN- γ -inducible protein (IP-10/CXCL10)	mRNA, protein	–	–
IFN-inducible T-cell alpha chemoattractant (I-TAC/CXCL11)	mRNA	–	–
Macrophage inflammatory protein 1 α	mRNA, protein	–	–
Monocyte chemoattractant protein 1 (MCP-1/CCL3)	Protein	–	–
Monokine induced by IFN- γ (MIG/CXCL9)	mRNA, protein	–	–
MCP-3 (CCL7)	mRNA	–	–
MCP-4 (CCL13)	mRNA	–	–
RANTES (CCL5)	mRNA, protein	72 $\pm$ 15 pg	Crystalloid granules (matrix) and small secretory vesicles
<i>Growth factors</i>			
Heparin-binding epidermal growth factor-like binding protein (HB-EGF-LBP)	mRNA	–	–
Nerve growth factor	mRNA, protein	4 $\pm$ 2 pg	–
Platelet-derived growth factor, B chain	mRNA	–	–
Stem cell factor	mRNA, protein	–	Membrane, cytoplasm
Transforming growth factor α	mRNA, protein	22 $\pm$ 6 pg	Crystalloid granules (matrix) and small secretory vesicles
Transforming growth factor β 1	mRNA, protein	–	–

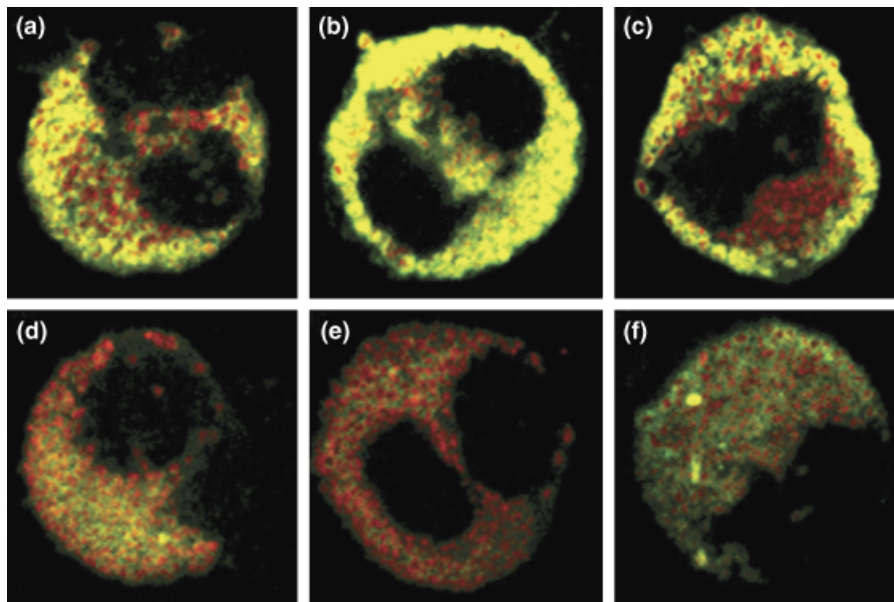


Fig. 12.1 Human eosinophils stimulated with interferon (IFN)- γ to induce rapid release of RANTES. Cells were stained with antibody to major basic protein (MBP) (red) and antibody to RANTES (green) in a time course study of RANTES mobilization. Yellow indicates colocalization of MBP and RANTES to similar granule compartments, which become distinct as early as 10 min following stimulation with IFN- γ (500 U/mL). Unstimulated cells (a) were compared with cells stimulated for (b) 5 min, (c) 10 min, (d) 30 min, (e) 60 min, and (f) 16 hours. Original magnification $\times 1000$.

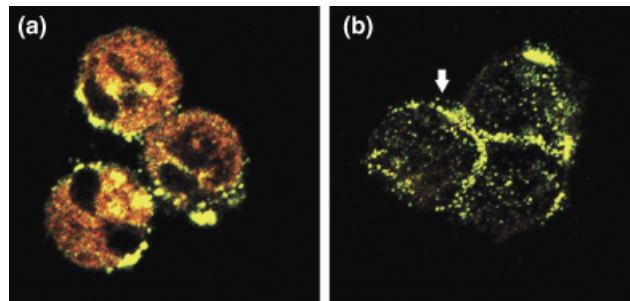


Fig. 12.2 Translocation of VAMP-2 during interferon (IFN)- γ stimulation of human eosinophils. Immunolabeling for VAMP-2 (green) showed moderate colocalization with RANTES (red) in (a) unstimulated eosinophils, but these were strongly colocalized at the cell membrane (arrow) following 5 min of stimulation with IFN- γ 500 U/mL (b). Original magnification $\times 1000$.

SNAREs can occur via clostridial neurotoxins containing zinc endopeptidase activity, particularly tetanus toxin and botulinum toxin serotypes (BoNT/A, B, C, D, E, F, and G). These toxins have been used to characterize the dependency of secretion on SNARE complex formation.

Many SNARE proteins have also been identified in nonneuronal secretory cells including syntaxin-4 and SNAP-23 (Ravichandran *et al.* 1996), while VAMP-2 expression is distributed between neuronal and nonneuronal tissues (Rossetto *et al.* 1996). In addition, VAMP-4 (Steegmaier *et al.* 1999), VAMP-5 (Zeng *et al.* 1998), and the tetanus toxin-insensitive proteins VAMP-7 (formerly known as tetanus toxin-insensitive VAMP or TI-VAMP)

(Galli *et al.* 1998; Advani *et al.* 1999; Hibi *et al.* 2000; Ward *et al.* 2000) and VAMP-8 have been characterized in nonneuronal tissues (Mullock *et al.* 2000; Paumet *et al.* 2000; Polgar *et al.* 2002).

Eosinophils express VAMP-2, VAMP-7, VAMP-8, syntaxin-4, and SNAP-23 (Feng *et al.* 2001; Lacy *et al.* 2001; Logan *et al.* 2002, 2003, 2006), whereas they do not contain detectable levels of the classical neuronal SNARE proteins (syntaxin-1, SNAP-25, and VAMP-1) (Lacy *et al.* 1995). Eosinophil VAMP-2 is expressed in a population of small secretory vesicles that store the chemokine RANTES, which translocates to the cell membrane during IFN- γ stimulation (Fig. 12.2) (Lacy *et al.*

1999, 2001). Immunofluorescence staining showed that syntaxin-4 and SNAP-23 were localized to the cell membrane in eosinophils, where they may function as

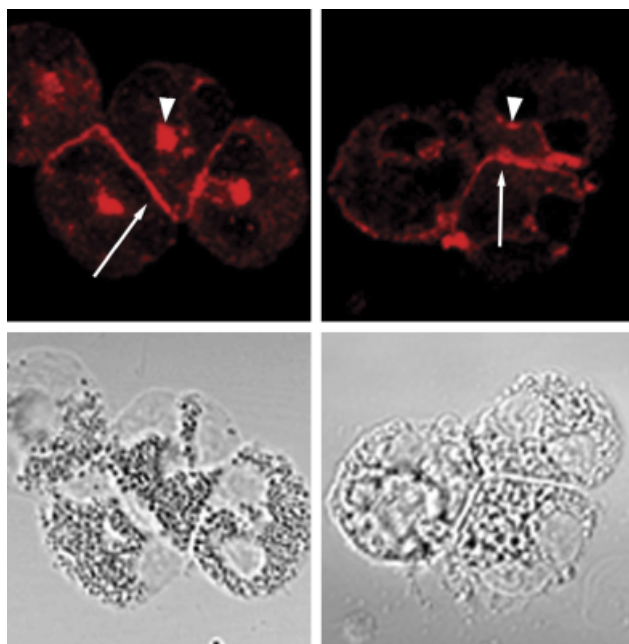


Fig. 12.3 Localization of SNAP-23 and syntaxin-4 in eosinophils. (*Left panel*) Distribution of SNAP-23 at cell membranes (arrow) as well as at an intracellular site that colocalizes with the Golgi apparatus (arrowhead). (*Right panel*) Antibody to syntaxin-4 shows expression in the cell membrane (arrow) and endoplasmic reticulum (arrowhead). Lower panels indicate differential interference contrast images. Original magnification $\times 630$.

cognate intracellular receptors for VAMP-2 (Fig. 12.3) (Logan *et al.* 2002). Inhibition of VAMP-2 binding led to the loss of IgE-induced ECP release in permeabilized eosinophils (Hoffmann *et al.* 2001). A novel isoform of VAMP, known as tetanus-insensitive VAMP (TI-VAMP, also known as VAMP-7), is a putative vesicular SNARE isoform for regulation of lysosomal fusion (Advani *et al.* 1999; Ward *et al.* 2000; Rao *et al.* 2004). VAMP-7 and VAMP-8 are abundantly expressed in eosinophil crystalloid granules, while VAMP-7 was shown to be required for exocytosis of crystalloid granule as well as small secretory vesicles (Logan *et al.* 2006). This finding suggests that VAMP-2 and VAMP-7 may play overlapping roles in the release of small secretory vesicles from eosinophils, although their release may be more dependent on VAMP-7 (Fig. 12.4). In summary, SNARE isoforms may play a crucial role in the regulation of granule fusion in eosinophils.

EOSINOPHIL SURFACE MARKERS

The first characterization of eosinophil surface molecules demonstrated that eosinophils express a large number of cell-surface markers including adhesion molecules, apoptotic signaling molecules, chemokine, complement and chemotactic factor receptors, cytokine receptors, and immunoglobulin receptors (Gupta *et al.* 1976; Ebisawa *et al.* 1995). Since these studies and the discovery of new immune receptors (Toll-like receptors, inhibitory

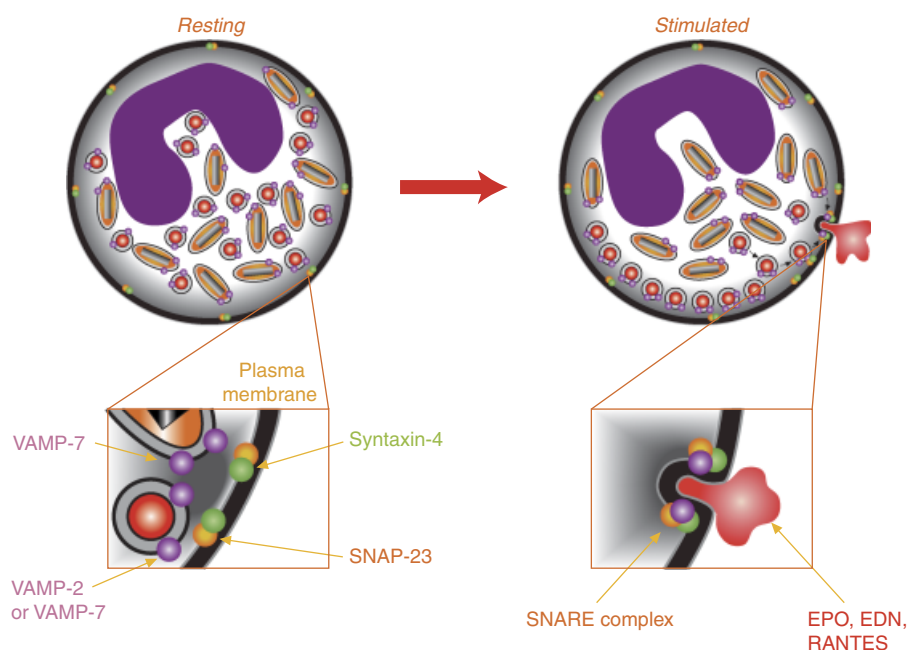


Fig. 12.4 Scheme showing SNARE-dependent exocytotic pathways for crystalloid granules and secretory vesicles in eosinophils.

receptors and Siglecs) and development of new reagents, the list has been extended revealing that eosinophils express an array of surface structures that were previously thought to be exclusively expressed by other cell types (Tachimoto & Bochner 2000; Rothenberg & Hogan 2006).

Adhesion molecules

Transmigration of the eosinophil through the vascular endothelium is a multistep process involving rolling, tethering, firm adhesion, and transendothelial migration (Wardlaw *et al.* 1994; Wardlaw 2000). The initial steps of eosinophil rolling and tethering are regulated by selectins and their counterligands expressed on the endothelium (Ebnet *et al.* 1996; Wardlaw 1999). Eosinophils have been shown to constitutively express L-selectin, which regulates eosinophil rolling on the endothelium *in vivo* (Georas *et al.* 1992; Sriramarao *et al.* 1994). Ligands for L-selectin include CD34 and MAdCAM-1 expressed endothelium (Berg *et al.* 1993). Eosinophils also express CD162 (P-selectin glycoprotein ligand-1 or PSGL-1) and sialyl-Lewis x (CD15 s), which interact with E-selectin and P-selectins and regulate eosinophil tethering to endothelium (Symon *et al.* 1996). The firm adhesion of the eosinophil, and transmigration across the vascular epithelium into tissues is regulated by coordinated interaction between networks involving chemokine and cytokine signaling, eosinophil adhesion molecules (e.g., selectins and integrins), and integrin receptors such as vascular cell adhesion molecule (VCAM)-1, mucosal addressin cell adhesion molecule (MAdCAM)-1 and intercellular adhesion molecule (ICAM)-1 expressed on vascular endothelial cells (Kunkel & Butcher 2002; Hogan *et al.* 2004). Integrins are heterodimeric surface molecules consisting of an α and β chain and eosinophils express members of the $\beta 1$ ($\alpha 4\beta 1$ and $\alpha 6\beta 1$), $\beta 2$ ($\alpha L\beta 2$, $\alpha M\beta 2$, $\alpha X\beta 2$, and $\alpha D\beta 2$) and $\alpha 7$ ($\alpha 4\beta 7$) integrin families (Georas *et al.* 1993; Grayson *et al.* 1998; Tachimoto & Bochner 2000; Bochner & Schleimer 2001; Tachimoto *et al.* 2002). These various integrin molecules selectively interact with adhesion receptors (VCAM-1, MAdCAM-1, ICAM-1, -2 and -3, and fibrinogen) expressed on the vascular endothelium.

The specific interaction of cell-surface integrins with adhesion receptors (VCAM-1, MAdCAM-1, ICAM-1, ICAM-2, ICAM-3, and fibrinogen) facilitates eosinophil migration into various tissue compartments during inflammation. For example, eosinophil recruitment to the site of allergic inflammation in the lung and skin is

regulated by VLA-4 ($\alpha 4\beta 1$ integrin)/VCAM-1-dependent processes (Weg *et al.* 1993; Abraham *et al.* 1994; Nakajima *et al.* 1994; Pretolani *et al.* 1994; Gonzalo *et al.* 1996). Pretreatment of mice with neutralizing monoclonal antibodies against $\alpha 4$ or $\beta 1$ integrin or genetic deletion of VCAM-1 attenuates eosinophil accumulation in the lung during allergic airways disease (Weg *et al.* 1993; Abraham *et al.* 1994; Nakajima *et al.* 1994; Pretolani *et al.* 1994; Gonzalo *et al.* 1996). Recent experimental studies have demonstrated that eosinophil recruitment into different tissue compartments (gastro-intestinal tract) is regulated by differential adhesion pathways. For example, eotaxin-1-dependent eosinophil recruitment to the small intestine is MAdCAM-1/ $\alpha 4\beta 7$ integrin dependent (Mishra *et al.* 2002), whereas eosinophil accumulation in the colon is regulated by a $\beta 2$ integrin pathway (ICAM-1) and can occur independently of $\alpha 4$ and $\beta 7$ integrin-independent pathways (Forbes *et al.* 2006).

Chemokine, complement and other chemotactic factor receptors

Experimental investigations have shown eosinophils to constitutively express the chemokine receptors CCR3 and CCR1 (Ponath *et al.* 1996; Phillips *et al.* 2003; Elsner *et al.* 2005). Consistent with this observation, eosinophils respond to CCR1 and CCR3 ligands including macrophage inflammatory protein (MIP)-1 α /CCL3, RANTES/CCL5, macrophage chemotactic protein (MCP)-2/CCL8, MCP-3/CCL7 and MCP-4/CCL-13, eotaxin-1/CCL11, eotaxin-2/CCL24 and eotaxin-3/CCL26, and mucosa-associated epithelial chemokine (MEC)/CCL28. Eosinophils have also been shown to express a number of other chemokine receptors including CXCR3, CXCR4, CCR5, CCR6, and CCR8 following activation by IL-5 (Sullivan *et al.* 1999; Nagase *et al.* 2000; Oliveira *et al.* 2002). While chemokines are thought to primarily regulate the migration pattern of eosinophils, they have also been shown to promote eosinophil activation and function (Zimmermann *et al.* 2003). For example, RANTES/CCL5 and eotaxin-1/CCL11 have been shown to promote cellular activation and modulate respiratory burst in eosinophils (Elsner *et al.* 1997, 1999).

Cytokine receptors

Three cytokines, IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF), are particularly important in regulating eosinophil development, and

eosinophils have been shown to express the specific cytokine receptor subunit for IL-3 (IL-3R α , CD123), IL-5 (IL-5R α , CD125) and GM-CSF (GM-CSFR α , CD116) and also the shared β chain (CD131) (Lopez *et al.* 1986, 1988; Rothenberg *et al.* 1988; Takatsu *et al.* 1994). Functional studies have demonstrated that cytokines including stem cell factor (SCF), IFN- γ , tumor necrosis factor (TNF)- α , IL-4, and IL-9 activate eosinophil functions, suggesting that eosinophils express the c-kit receptor (CD117), IFN- γ R α -chain (CDw119), TNF- α receptor types 1 and 2 (CD120a, CD120b), type 1 IL-4 receptor [IL-4R α -chain (CD124) and the common α -chain (CD132)], and the IL-9 receptor [IL-9R α -chain (CD129)/CD132] (Wallen *et al.* 1991; Yuan *et al.* 1997; Dubois *et al.* 1998; Nutku *et al.* 1999; Hauber *et al.* 2004). Consistent with these observations, both the type 1 (CD120a) and type 2 (CD120b) TNF receptors have been identified on human eosinophils by fluorescence-activated cells sorting analysis and immune electron microscopy. Activation of these receptors are thought to promote eosinophil apoptosis (Zeck-Kapp *et al.* 1994; Zeck-Kapp & Kapp 1995). While eosinophils have been shown to express a number of IFN receptor superfamily members, including receptors for IFN- α , IFN- β , IFN- γ , and IL-10 (Giembycz & Lindsay 1999), only the receptor for IFN- γ (type 2 IFN) has been convincingly identified (Aldebert *et al.* 1996; Ishihara *et al.* 1997; Matsuyama *et al.* 1998; Ochiai *et al.* 1999). The IL-2 receptor is composed of three polypeptide chains, an α -chain (p55) (CD25), β chain (p75) (CD122), and a γ chain (CD132) that is common to several other cytokine receptors. Experimental investigations have demonstrated that IL-2 induces eosinophil chemotaxis, suggesting that the cognitive receptor for the cytokine was expressed on the eosinophils. This was confirmed by the observation that the IL-2-mediated effects could be blocked by antibodies against IL-2R α chain (p55) and β chain (p75) (Rand *et al.* 1991).

Complement receptors

Initial studies suggested that eosinophils express complement receptors for C3a and C5a (Daffern *et al.* 1995; DiScipio *et al.* 1999); however, more recent studies have revealed that eosinophils also express CR1 (CD35), CR3 (CD11b/CD18), CR4 (CD11c), CD103, and receptors for C1q (Walsh *et al.* 1990; Giembycz & Lindsay 1999). CR1 is recognized by the complement fragments C3b, C4b, iC3b, and C1q. The expression of CR1 on eosinophils is regulated by certain stimuli, including LTB₄, 5-HETE, and 5-HPETE (Fischer *et al.* 1986). CR3 has also been

shown to be expressed on eosinophils: CR3 interacts with a number of ligands including iC3b and ICAM-1, all of which could activate eosinophils resulting in eosinophil priming and degranulation (Koenderman *et al.* 1991).

Prostaglandin and leukotriene receptors

Clinical and experimental studies have demonstrated that eosinophils express both cysteinyl leukotriene receptors (CysLT1R and CysLT2R), high-affinity prostaglandin (PG)D₂ type 2 receptor, and the PAF receptor (Wang *et al.* 1999; Fujii *et al.* 2005; Zinchuk *et al.* 2005). Interestingly, the PGE₂ receptor is also expressed by basophils and Th2 cells (and is now designated "chemoattractant receptor Th2 cells" or CRTH2) and appears to mediate Th2 cell and eosinophil/basophil recruitment (Hirai *et al.* 2001). Both mature eosinophils and immature eosinophil progenitors express CysLT1R, whereas CysLT2R has only been identified on mature eosinophils. Expression of these receptors has been shown to be upregulated on eosinophils from asthmatics during exacerbations (Fujii *et al.* 2005). Notably, CysLT2R expression on eosinophils was selectively greater in nonatopic asthmatics (Fujii *et al.* 2005). The function of these receptors on eosinophils has not yet been fully defined. However, leukotrienes (LTB₄, LTD₄, LTE₄), PAF, and 5-oxo-6,8,11,14-eicosatetraenoic acid induce eosinophil recruitment, suggesting that they may regulate eosinophil transmigration (Powell *et al.* 1995; Bandeira-Melo *et al.* 2000; Ohshima *et al.* 2002; Shiraishi *et al.* 2005). Previous reports have also demonstrated that *in vitro* suppression of cysteinyl leukotriene activity by a CysLT1R antagonist blocks eosinophil differentiation and/or maturation, suggesting that cysteinyl leukotrienes may play a role in eosinophil lineage commitment and maturation (Thivierge *et al.* 2000). Cysteinyl leukotrienes have also been shown to promote eosinophil release of cytokines including IL-4 (Bandeira-Melo *et al.* 2002a,b). Eosinophils have also been shown to express high levels of the histamine H₄ receptor that mediates eosinophil chemoattraction and activation *in vitro* (O'Reilly *et al.* 2002).

Immunoglobulin receptors

Eosinophils express Fc receptors for IgA, IgD, IgG, and IgM (Giembycz & Lindsay 1999). CD32 (Fc γ RII) is constitutively expressed on resting human eosinophils (Hartnell *et al.* 1990), and is upregulated by IFN- γ (Hartnell *et al.* 1992). These receptors not only function

as IgG receptors but also appear to have a role in stimulating eosinophil survival, degranulation, and generation of leukotrienes (Cromwell *et al.* 1988, 1990; Kita *et al.* 1991; Kim *et al.* 1999). Eosinophils do not constitutively express Fc γ RI (CD64) or low-affinity Fc γ RIII (CD16), although expression can be upregulated by cytokines, IFN- γ , complement (C5a), and PAF (Hartnell *et al.* 1992). Eosinophils do appear to express IgA receptors (CD89) (Monteiro *et al.* 1993). *Ex vivo* studies have demonstrated that eosinophil degranulation can be induced by IgA-coated particles, suggesting that IgA-receptor interaction induces eosinophil degranulation (Abu Ghazaleh *et al.* 1989). The expression or presence of the low-affinity IgE receptor (CD23) or the high-affinity IgE receptor on eosinophils remains controversial (Kita & Gleich 1997). Some studies suggest that eosinophils bind to IgE (Capron *et al.* 1985, 1995), although more recent investigations suggest that eosinophils express little if any α or β chains for the high-affinity receptor or the low-affinity CD23 IgE receptor (Ying *et al.* 1998; Kita *et al.* 1999; Seminario *et al.* 1999).

Other cell-surface structures

Eosinophil apoptosis has been shown to be induced by two surface structures, CD95 (first) and CD69 (Matsumoto *et al.* 1995; Walsh *et al.* 1996). Eosinophils also to express CD9, CD37, CD52, CD63, CD81, CD82, and CD151 (Ebisawa *et al.* 1995). Experimental investigations have demonstrated that eosinophils can express antigen to naive CD4⁺ T cells and promote T-cell proliferation and polarization (Shi *et al.* 2000, 2004; Shi 2004). Consistent with this observation, eosinophils have also been shown to express MHC class II and the necessary costimulatory signals for T-cell activation and proliferation, including the type 1 interval surface membrane glycoprotein CD40 as well as CD80 and CD86 (Ohkawara *et al.* 1996; Woerly *et al.* 1999). The expression of CD80 and CD86 appears to be independently regulated by IL-3 and GM-CSF (Tamura *et al.* 1996). Human eosinophils have also been shown to express the MHC class II protein human leukocyte antigen (HLA)-DR (Shi 2004). Interestingly, peripheral eosinophils of most normal eosinophilic donors do not express HLA-DR proteins, although sputum eosinophils and bronchoalveolar lavage (BAL) eosinophils from asthmatics have been shown to express HLA-DR (Hansel *et al.* 1991; Sedgwick *et al.* 1992). Notably, levels of HLA-DR expression on BAL eosinophils from allergic subjects following segmental challenge were elevated compared with peripheral blood eosinophils,

suggesting that recruitment and activation of eosinophils following allergen challenge promotes HLA-DR expression (Sedgwick *et al.* 1992). HLA-DR expression on eosinophils has been shown to be regulated by IL-3, IL-4, GM-CSF, and IFN- γ (Lucey *et al.* 1989; Weller *et al.* 1993).

Inhibitory receptors

The CD2 subfamily of the IgE superfamily includes CD2, CD48 (BLAST1) and BTM1 (CD58), LFA3, CD84, IL-9b, CD150, CD229, and 2B4 (CD244). Recent studies have demonstrated that eosinophils express the CD2 subfamily of receptors, namely CD48 and 2B4 (CD244) (Munitz *et al.* 2005). CD48 is a glycosylphosphatidylinositol (GPI)-anchored protein involved in cellular activation, costimulation, and adhesion. CD48 expression is elevated on human eosinophils from atopic asthmatics and is upregulated by IL-3 (Munitz *et al.* 2006). Cross-linking of CD48 on eosinophils triggers eosinophil degranulation (Munitz *et al.* 2005, 2006). Notably, CD48 is the high-affinity ligand for 2B4. Eosinophils also express inhibitory receptor IRp60/CD300a, p140, Siglec-8, Siglec-10, ILT5/LIR3, CD33, and p75/adhesion inhibitory receptor molecules (Munitz & Levi-Schaffer 2007). IRp60 activation has been shown to be involved in the suppression of eosinophil activation (Munitz *et al.* 2005). Interestingly, CCR3 has been shown to induce negative signaling in murine eosinophils following receptor engagement with the Th1 chemokine CXCL9 (Mlg) (Fulkerson *et al.* 2005).

Toll-like receptors

Eosinophils express mRNA for a number of Toll-like receptors (TLR) including TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, and TLR10 (Plotz *et al.* 2001; Sabroe *et al.* 2002; Nagase *et al.* 2003). The level of TLR expression on eosinophils is low relative to other granulocytes such as neutrophils, except for relatively elevated levels of TLR7/TLR8 (Nagase *et al.* 2003). The natural ligands for TLR7/TLR8 are currently not clear, although significant evidence suggests that guanosine- and uridine-rich ssRNA are physiologic ligands for these TLRs (Heil *et al.* 2004). Functional analysis using TLR-specific ligands reveals that TLR7/TLR8 ligands (R-848) induced eosinophil activation (superoxide production) and prolonged eosinophil survival. The expression of TLR7/TLR8 has been shown to be regulated by cytokines including IFN- γ (Nagase *et al.* 2003).

EOSINOPHILS AND IMMUNE REGULATION

In recent years, eosinophils have been shown to possess the ability to perform numerous immune functions, including antigen presentation (Shi *et al.* 2000; MacKenzie *et al.* 2001) and exacerbation of inflammatory responses through their capacity to release a range of largely preformed cytokines and lipid mediators (Gleich & Adolphson 1986; Weller 1994).

Thymic eosinophils

Eosinophils transmigrate into the thymus during the neonatal period, reaching maximum levels by 2 weeks of age. Interestingly, their absolute levels are approximately equivalent to that of thymic dendritic cells (Throsby *et al.* 2000). Eosinophils primarily localize to the corticomedullary region of the thymus and reach basal levels by 28

days of age. Subsequently, an increase in thymic eosinophil levels at 16 weeks of age corresponds to the commencement of thymic involution. Notably, eosinophils at this stage localize to the medullary region.

Thymic eosinophils express high levels of MHC class II molecules and moderate levels of MHC class I and the costimulatory molecules CD86 (B7.2) and CD30L (CD153) (Fig. 12.5). Furthermore, thymic eosinophils are CD11b/CD11c double-positive and appear to be activated, as they lose expression of GL-1 and CD62L and upregulate CD25 and CD69 surface expression. Analysis of thymic eosinophil cytokine production reveals that eosinophils express mRNA for the proinflammatory cytokines TNF- α , TGF- β , IL-1 α and IL-6 and the Th2 cytokines IL-4 and IL-13 (Throsby *et al.* 2000). Notably, the recruitment of eosinophils into the thymus is regulated by eotaxin-1, which is constitutively expressed in the thymus (Matthews *et al.* 1998).

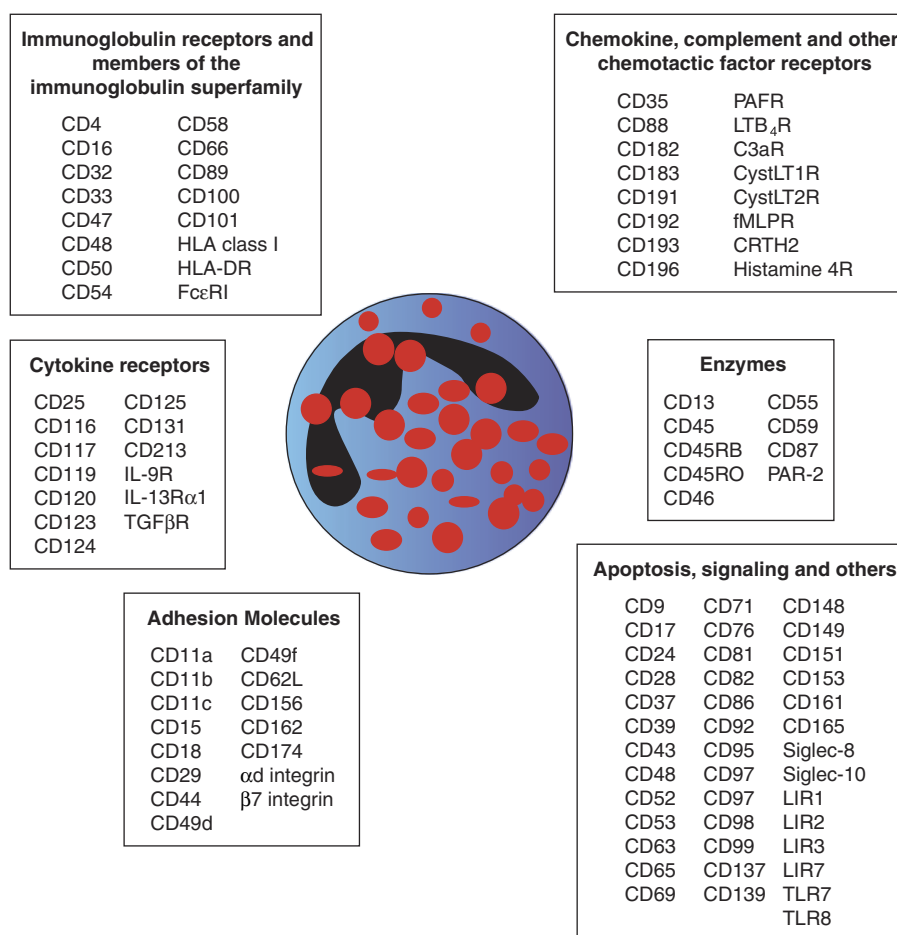


Fig. 12.5 Schematic diagram showing surface molecules expressed by human eosinophils. Molecules have been listed based on convincing evidence for their expression as assessed by flow cytometry or inferred by cellular responsiveness to specific stimuli. Cluster designation (CD) for particular molecules is indicated based on the most recent classification (www.ncbi.nlm.nih.gov/prov/).

It has been postulated that eosinophils are associated with MHC class I-restricted thymocyte deletion. Consistent with this notion, the biphasic recruitment of eosinophils and their anatomic localization within discrete compartments of the thymus coincides with negative selection of double-positive thymocytes (Throsby *et al.* 2000). Employing an experimental model of acute negative selection, increased thymic eosinophil levels have been demonstrated in MHC class I-restricted female H-Y T-cell receptor transgenic mice following cognate peptide injection. In addition, eosinophils are associated with clusters of apoptotic bodies, suggesting eosinophil-mediated MHC class I-restricted thymocyte deletion. Thymic eosinophils have the capacity to promote thymocyte apoptosis as they express costimulatory molecules involved in clonal deletions, such as CD30L (CD153) and CD66 (Throsby *et al.* 2000). Additionally, eosinophils may induce thymocyte apoptosis via free radicals, as thymic eosinophils express high levels of NADPH oxidase activity; notably, developing thymocytes have increased sensitivity to free radicals due to downregulation of $\text{Cu}^{2+}/\text{Zn}^{2+}$ superoxide dismutase.

Antigen presentation

Recent clinical and experimental investigations have shown that eosinophils can function as antigen-presenting cells (Fig. 12.6). Eosinophils are capable of processing and presenting a variety of microbial, viral, and parasitic antigens, as well as superantigens (Shi 2004). GM-CSF-treated eosinophils promote T-cell proliferation in response to staphylococcal superantigen (*Staphylococcus enterotoxins A, B and E*) stimulation (Mawhorter *et al.* 1994). Furthermore, eosinophils incubated with human rhinovirus-16 promote rhinovirus-16-specific T-cell proliferation and IFN- γ secretion (Handzel *et al.* 1998). Eosinophils can also effectively present soluble antigens to CD4^{+} T cells, thereby promoting T-cell proliferation and polarization. Adoptive transfer of antigen-pulsed eosinophils results in eosinophil-dependent T-cell proliferation (MacKenzie *et al.* 2001). Furthermore, addition of antigen to eosinophil and T-cell cocultures promotes heightened T-cell proliferative responses (Shi *et al.* 2000). The capacity of eosinophils to present antigen has been debated in some publications. It is interesting to note that the failure of eosinophils to present antigen may be related to the methods used for isolating eosinophils. For example, lysis of erythrocytes with ammonium chloride, an inhibitor of lysosome acidification (needed for antigen presentation), nega-

tively correlates with eosinophil antigen presentation activity (Shi *et al.* 2000; van Rijt *et al.* 2003).

Eosinophils secrete an array of cytokines capable of promoting T-cell proliferation, and activation of Th1 or Th2 polarization (IL-2, IL-4, IL-6, IL-12, IL-10) (Kita 1996; Lacy & Moqbel 2000; Shi *et al.* 2000; MacKenzie *et al.* 2001) (Fig. 12.6 and Table 12.2). Recent attention has been drawn to the ability of murine eosinophils to produce IL-4. Employing mice with enhanced green fluorescent protein (GFP) in the IL-4 gene locus (4get mice), eosinophils appear to be a primary source of IL-4 following parasitic infection or anti-IgD treatment (a strong Th2 stimulator). Notably, while the IL-4 gene locus is transcriptionally active in eosinophils, the amount of IL-4 protein production appears to be lower than in T cells and basophils (Shinkai *et al.* 2002; Khodoun *et al.* 2004; Voehringer *et al.* 2004). Furthermore, murine eosinophils promote IL-4, IL-5, and IL-13 secretion by CD4^{+} T cells (MacKenzie *et al.* 2001). Eosinophils can also regulate T-cell polarization through their synthesis of indoleamine 2,3-dioxygenase, an enzyme involved in oxidative metabolism of tryptophan, converting tryptophan to kynurenines. Kynurenines regulate Th1 and Th2 imbalance by promoting Th1 cell apoptosis (Odemuyiwa *et al.* 2004). The eosinophil-mediated T-cell proliferative and cytokine secretion responses are dependent on costimulation. Indeed, blockade of CD80, CD86, and CTLA-4 by neutralizing antibodies inhibits eosinophil-elicited T-cell proliferation and cytokine secretion (Shi 2004).

Fluorescent labeling studies have revealed that eosinophils instilled into the trachea of mice traffic into the draining peritracheal lymph nodes and localize to the T-cell-rich paracortical regions (B-cell zones) within 24 hours (Shi *et al.* 2000). Employing models of allergic airway disease and gastrointestinal allergy, investigators have demonstrated that inhalation of antigen promotes eosinophil homing to the draining endotracheal lymph nodes and Peyer's patches (Korsgren *et al.* 1997; Mishra *et al.* 2000; Hogan *et al.* 2001; MacKenzie *et al.* 2001).

Interestingly, a recent investigation suggests that eosinophils can only promote proliferation of effector T cells but not naive T cells (van Rijt *et al.* 2003). Moreover, eosinophils pulsed with ovalbumin peptide and cocultured with ovalbumin-specific T-cell receptor transgenic T cells (D011.10T cells) induced effector T-cell proliferation; however, when cocultured with naive CD4^{+} T cells, no T-cell proliferation was observed. It is tempting to speculate that eosinophils traffic to draining lymph

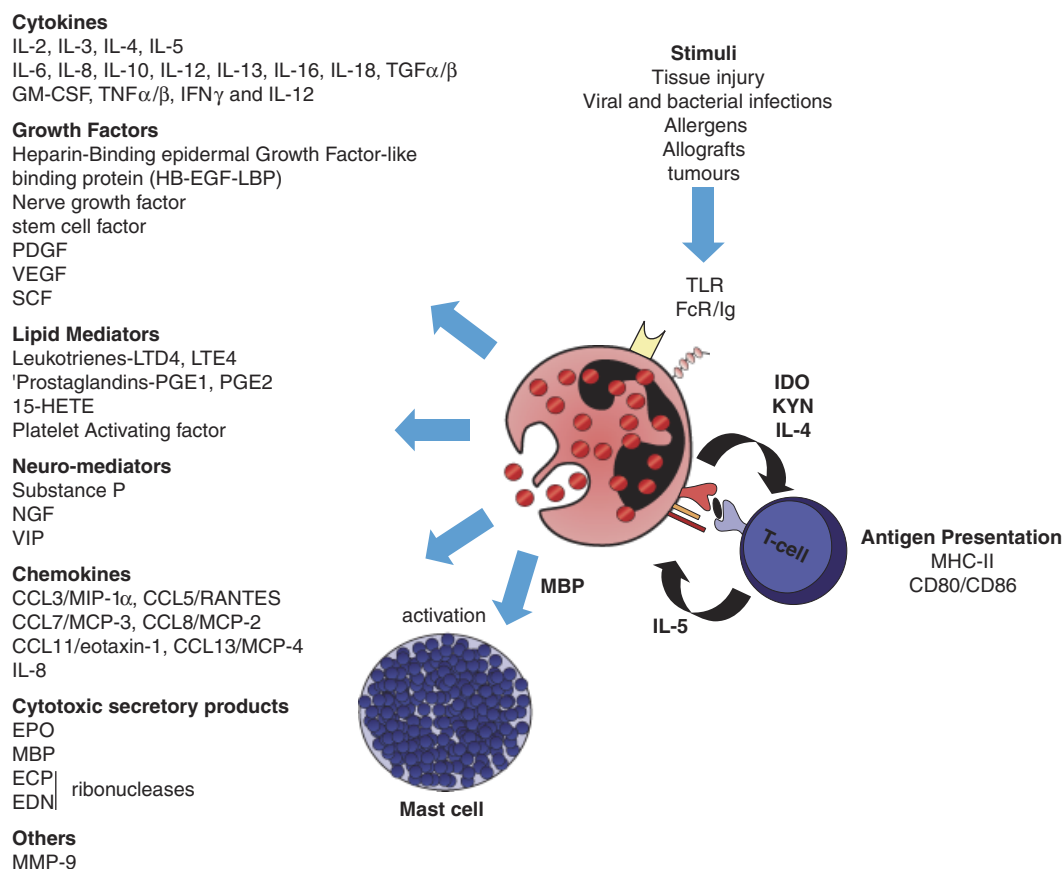


Fig. 12.6 Schematic diagram of an eosinophil and its multifunctional effects. Eosinophils are bilobed granulocytes with eosinophilic staining secondary granules. The secondary granules contain four primary cationic proteins designated eosinophil peroxidase (EPO), major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil derived neurotoxin (EDN). All four proteins are cytotoxic molecules; in addition, ECP and EDN are ribonucleases. Eosinophils respond to diverse stimuli including nonspecific tissue injury, viral infections, allografts, allergens, and tumors. In addition to releasing their preformed cationic proteins, eosinophils can also release a variety of cytokines, chemokines, lipid mediators, and neuromodulators. Eosinophils directly communicate with T cells and mast cells in a bidirectional manner. Eosinophils activate T cells by serving as antigen-presenting cells and eosinophil-derived MBP is a mast cell secretagogue. Eosinophils can also regulate T-cell polarization through synthesis of indoleamine 2,3-dioxygenase (IDO), an enzyme involved in oxidative metabolism of tryptophan, catalyzing the conversion of tryptophan to kynurenines (KYN), a regulator of Th1/Th2 balance. 15-HETE, 15-hydroxyeicosatetraenoic acid; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; NGF, nerve growth factor; PDGF, platelet-derived growth factor; RANTES, regulated on activation normal T-cell expressed and secreted; SCF, stem cell factor; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal polypeptide. See text for definition of other abbreviations. (Adapted from Rothenberg & Hogan 2006 with permission.)

nodes in order to recruit activated effector T cells and promote proliferation of effector T cells.

Mast cell regulation

A substantial body of literature has emerged demonstrating that eosinophils have the capacity to regulate mast cell function (see Fig. 12.6). Notably, human umbilical cord blood-derived mast cells can be activated by MBP to release histamine, PGD₂, GM-CSF, TNF- α , and IL-8 (Piliponsky *et al.* 2002). The activation of mast cells by MBP elicits not only exocytosis but also eicosanoid generation and cytokine production, both of which are

prominent responses following Fc ϵ RI-dependent activation of mast cells (Piliponsky *et al.* 2002). Incubation of rat peritoneal mast cells with native MBP, EPO, and ECP (but not EDN) results in concentration-dependent histamine release (Zheutlin *et al.* 1984). Several studies have shown that MBP induces mast cell activation via a similar pathway to that observed with other polybasic compounds such as substance P, compound 48/80, and bradykinin (Piliponsky *et al.* 2001). Freshly isolated human lung mast cells are resistant to IgE-independent activation; however, highly purified lung mast cells cocultured with human lung fibroblasts are sensitive to IgE-independent activation by MBP (Piliponsky *et al.*

2002). Interestingly, activation of eosinophils with the mast cell protease chymase promotes production of eosinophil-derived stem cell factor, a critical mast cell growth factor. Eosinophils also produce nerve growth factor (NGF) (Solomon *et al.* 1998), a cytokine not only involved in survival and functional maintenance of sympathetic neurons but also in immune regulation. For example, NGF promotes mast cell survival and activation (Horigome *et al.* 1994; Bullock & Johnson 1996). NGF is preformed in eosinophils and acts in an autocrine fashion by activating release of EPO (Solomon *et al.* 1998). EPO activates rat peritoneal muscles to release histamine, suggesting a role of eosinophil-derived NGF in mast cell–eosinophil interactions. Thus, eosinophils and mast cells communicate in a bidirectional fashion.

EOSINOPHIL DEVELOPMENT

Eosinophils are produced in the bone marrow from pluripotent stem cells, which first differentiate into a hybrid precursor with shared properties of basophils and eosinophils, and then into a separate eosinophil lineage (Boyce *et al.* 1995). Initial studies examining the *eos47* gene (encoding EOS47, the avian ortholog of the mammalian melanotransferrin gene), a gene specifically expressed by bone marrow eosinophils, revealed that a 309-bp promoter region consisting of binding sites for Myb-, Ets-, c/EBP-, and GATA-type transcription factors were responsible for governing lineage-specific expression (McNagny *et al.* 1998).

More recent investigations have supported these initial observations, demonstrating that eosinophil lineage specification is dictated by the interplay of at least three classes of transcription factors including GATA-1 (a zinc finger family member), PU.1 (an Ets family member), and c/EBP members (CCAAT/enhancer-binding protein family) (Nerlov & Graf 1998; Nerlov *et al.* 1998; McNagny & Graf 2002). Interestingly, these three transcription factors are expressed in a variety of hematopoietic lineages, although their synergistic mechanism of action in eosinophils promotes lineage specificity. The expression level of PU.1 specifies distinct cell lineage fates, with low levels specifying lymphocytic and high levels myeloid differentiation (De Koter & Singh 2000; Du *et al.* 2002). In most cell types, GATA-1 and PU.1 antagonize each other, but have synergistic activity in regulating eosinophil lineage specification (and eosinophil granule protein transcription) (Du *et al.* 2002). The specificity of these factors for eosinophils is conserved across species, for example c/EBP factors and GATA-1 drive differentia-

tion of chicken progenitor cells into eosinophils (McNagny & Graf 2002). Of these transcription factors, GATA-1 is clearly the most important for eosinophil lineage specification as revealed by loss of the eosinophil lineage in mice harboring a targeted deletion of the high-affinity GATA-binding site in the GATA-1 promoter (Yu *et al.* 2002), and based on eosinophil differentiation experiments *in vitro* (Hirasawa *et al.* 2002). In particular, the specific activity of GATA-1 in eosinophils but not other GATA-1-positive lineages (mast cells, megakaryocytes, erythroid cells) appears to be mediated by a high-affinity palindromic (or “double”) GATA site (Du *et al.* 2002). This double GATA site is present in the downstream GATA-1 promoter and also in the regulatory regions of eosinophil-specific genes, including the eotaxin receptor CCR3, MBP, and the IL-5R α gene, and accounts for eosinophil-specific gene expression (Zimmermann *et al.* 2000a; Du *et al.* 2002; Yu *et al.* 2002). For example, the tandem double GATA site in the human MBP-P2 promoter is required for both promoter activity in human eosinophil cell lines and for synergistic transactivation by GATA-1 and PU.1 (Du *et al.* 2002). Previous studies have identified *cis*-acting sequences (*cis* elements) as important regulators of GATA-1 expression, particularly a 3-*cis*-acting sequence known as upstream enhancer HS1/G1HE (HS1) as a major enhancer of GATA-1 expression (McDevitt *et al.* 1997; Onodera *et al.* 1997). However, studies have demonstrated that HS1 deletion in mice does not affect eosinophil GATA-1 mRNA expression and eosinophil differentiation (Guyot *et al.* 2004).

A number of the c/EBP members have also been implicated in the regulation of eosinophil lineage commitment (Nerlov *et al.* 1998). Phenotypic characterization of c/EBP α -deficient mice revealed that these mice are devoid of eosinophils (Zhang *et al.* 1997). This is consistent with studies demonstrating eosinophil formation from cord blood progenitors by enforced expression of c/EBP α (Iwama *et al.* 2002). Collectively these studies suggest that coexpression of GATA-1 and c/EBP α are required for efficient eosinophil formation (Hirasawa *et al.* 2002; Iwama *et al.* 2002; McNagny & Graf 2002).

More recent investigations have demonstrated that expression of eosinophil granule proteins is also regulated by c/EBP ϵ and PU.1. Eosinophils from c/EBP ϵ ^{−/−} mice have an abnormal phenotype (Yamanaka *et al.* 1997). Notably, in these mice neutrophil secondary granule gene expression is severely impaired, suggesting that c/EBP ϵ may be involved in granule gene expression (Gombart *et al.* 2003). c/EBP ϵ and GATA-1 proteins have

been shown to weakly induce MBP expression; however, addition of PU.1 dramatically upregulates endogenous MBP expression, suggesting that PU.1 may regulate eosinophil-specific gene expression (Gombart *et al.* 2003). Consistent with these observations, MBP and EDN gene expression was attenuated in myeloid cell lines derived from PU.1^{-/-} mice. Furthermore, PU.1 has been shown to be involved in the expression of other eosinophil-specific gene expression including EDN (van Dijk *et al.* 1998). Granulocytes are generated from a small number of hematopoietic stem cells and this process is highly regulated and maintained at a constant level under steady-state conditions (Metcalf 1991; Tenen *et al.* 1997; Zhu & Emerson 2002). However, under conditions of inflammation or cytokine stimulation, termed "emergency granulopoiesis," the hematopoietic system greatly amplifies granulocyte formation. This process has been shown to be regulated by many cytokines (G-CSF, GM-CSF, IL-3). A recent study suggests that c/EBP factors (c/EBP α and c/EBP β) also play an important role in the regulation of different aspects of steady-state and inflammatory stimuli-induced (emergency) granulopoiesis (Kincade 2006). Employing c/EBP β -deficient mice, investigators have demonstrated normal steady-state granulopoiesis in the absence of c/EBP β , suggesting that c/EBP α is sufficient for steady-state granulopoiesis. This has been confirmed by the demonstration of complete loss of granulocytes in c/EBP α -deficient mice (Zhang *et al.* 1997). Notably, emergency granulopoiesis induced by cytokine stimulation was ablated in c/EBP β -deficient mice (Hirai *et al.* 2006), suggesting that c/EBP β selectively regulates emergency granulopoiesis. In support of this hypothesis, cytokine treatment induced c/EBP β but not c/EBP α or c/EBP ϵ transcripts in granulocyte progenitors. Furthermore, granulocytes can be generated from c/EBP α ^{-/-} progenitors following cytokine stimulation *in vivo* (Hirai *et al.* 2006). These studies suggest that c/EBP α regulates steady-state and c/EBP β emergency granulopoiesis (Hirai *et al.* 2006; Kincade 2006).

Three cytokines, IL-3, IL-5, and GM-CSF, are particularly important in regulating eosinophil development (Lopez *et al.* 1986, 1988; Rothenberg *et al.* 1988; Takatsu *et al.* 1994). These eosinophilopoietins likely provide permissive proliferative and differentiation signals following the instructive signals specified by the transcription factors GATA-1, PU.1, and c/EBPs. These cytokines are encoded by closely linked genes on chromosome 5q31. They bind to receptors that share a common β chain and have unique α chains (Vadas *et al.* 1994). Of

these three cytokines, IL-5 is the most specific to the eosinophil lineage and is responsible for selective differentiation of eosinophils (Sanderson 1992). IL-5 also stimulates the release of eosinophils from the bone marrow into the peripheral circulation (Collins *et al.* 1995). The critical role of IL-5 in the production of eosinophils is best demonstrated by genetic manipulation of mice. Overproduction of IL-5 in transgenic mice results in profound eosinophilia (Dent *et al.* 1990; Tominaga *et al.* 1991; Lee *et al.* 1997; Mishra *et al.* 2002) and deletion of the IL-5 gene causes a marked reduction of eosinophils in the blood and lungs after allergen challenge (Foster *et al.* 1996; Kopf *et al.* 1996). The overproduction of one or a combination of these three cytokines occurs in humans with eosinophilia, and diseases with selective eosinophilia are often accompanied by overproduction of IL-5 (Owen *et al.* 1989). The critical role of IL-5 in regulating eosinophils in humans has been demonstrated by several clinical trials with humanized anti-IL-5 antibody; this currently unapproved drug dramatically lowers eosinophil levels in the blood and to a lesser extent in the inflamed lung (Leckie *et al.* 2000; Flood-Page *et al.* 2003a; Kips *et al.* 2003).

EOSINOPHIL TRAFFICKING

Chemokine regulation of eosinophil and CD4⁺ T-cell trafficking

Under baseline conditions, most eosinophils traffic into the gastrointestinal tract where they normally reside within the lamina propria of all segments except the esophagus (Mishra *et al.* 1999). The gastrointestinal eosinophil is the predominant population of eosinophils. Under baseline conditions, eosinophil levels in the gastrointestinal tract occur independent of lymphocytes and enteric flora, indicating unique regulation compared with other leukocytes (Mishra *et al.* 1999). Indeed, the recruitment of gastrointestinal eosinophils is regulated by the constitutive expression of eotaxin-1, as demonstrated by the marked decrease of this population of eosinophils in eotaxin-1-deficient mice. The importance of eotaxin-1 in regulating the baseline level of eosinophils is reinforced by the observation that mice with targeted deletion of CCR3 (but not eotaxin-2-deficient mice) also have a deficiency in gastrointestinal eosinophils (Humbles *et al.* 2002; Pope *et al.* 2005a). In addition to trafficking into the gastrointestinal tract, under homeostatic conditions eosinophils home into the thymus, mammary gland, and uterus, also under the

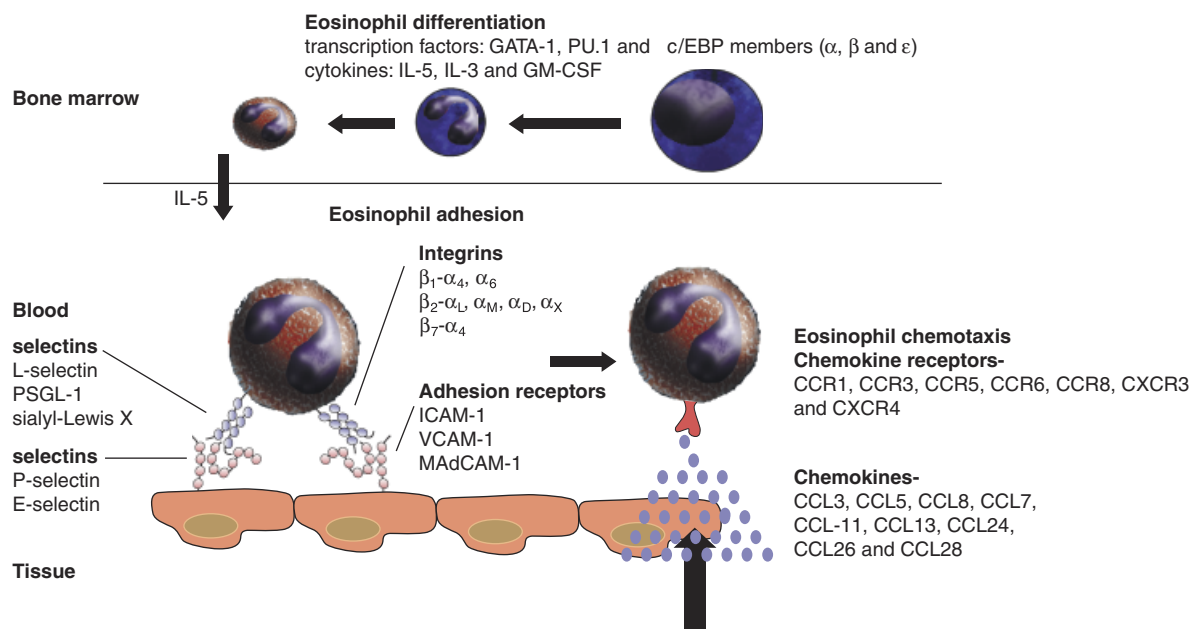


Fig. 12.7 Schematic representation of eosinophil trafficking. Eosinophils develop in the bone marrow where they differentiate from hematopoietic progenitor cells into mature eosinophils under the control of the critical transcription factors GATA-1, PU.1 and c/EBP members. The eosinophilopoietins IL-3, IL-5, and GM-CSF regulate eosinophil expansion, especially in conditions of hypereosinophilia. Eosinophil migration out of the bone marrow into the circulation is primarily regulated by IL-5. Circulating eosinophils subsequently interact with the endothelium by processes involving rolling, adhesion, and diapedesis. Depending on the target organ, eosinophils cross the endothelium into tissues by a regulated process involving coordinated interaction between networks involving the chemokines, eosinophil adhesion molecules, and adhesion receptors on the endothelium. (Adapted from Rothenberg & Hogan 2006 with permission.)

regulation of eotaxin-1 (Gouon-Evans *et al.* 2000; Rothenberg *et al.* 2001a). Notably, trafficking into the uterus is regulated by estrogen, as eosinophil and eotaxin-1 levels cycle with estrus (Gouon-Evans & Pollard 2001).

The trafficking of eosinophils into inflammatory sites has been shown to involve a number of cytokines (most notably the Th2 cell products IL-4, IL-5, and IL-13) (Sher *et al.* 1990a; Moser *et al.* 1992; Horie *et al.* 1997), adhesion molecules (e.g., β_1 , β_2 , and β_7 integrins) (Bochner & Schleimer 1994), chemokines such as RANTES and the eotaxins (Zimmermann *et al.* 2003), and other recently identified molecules (e.g., acidic mammalian chitinase) (Zhu *et al.* 2004) (Fig. 12.7). Of the cytokines implicated in modulating leukocyte recruitment, only IL-5 and the eotaxins selectively regulate eosinophil trafficking (Rankin *et al.* 2000). IL-5 regulates growth, differentiation, activation, and survival of eosinophils and has been shown to provide an essential signal for the expansion and mobilization of eosinophils from the bone marrow into the lung following allergen exposure (Collins *et al.* 1995). However, antigen-induced tissue eosinophilia can occur independent of IL-5, as demonstrated by residual tissue eosinophils in trials

using anti-IL-5 in patients with asthma (Flood-Page *et al.* 2003a), and by IL-5-deficient mice (Foster *et al.* 1996; Hogan *et al.* 1997). Recent studies have demonstrated an important role for the eotaxin subfamily of chemokines in eosinophil recruitment to the lung (Zimmermann *et al.* 2003).

Eotaxin was initially discovered using a biological assay in guinea pigs designed to identify the molecules responsible for allergen-induced eosinophil accumulation in the lungs (Jose *et al.* 1994; Rothenberg *et al.* 1995; Rankin *et al.* 2000). Subsequently, utilizing genomic analyses, two additional chemokine genes have been identified in the human genome that encode CC chemokines with eosinophil-selective chemoattractant activity, and have hence been designated eotaxin-2 and eotaxin-3 (Zimmermann *et al.* 2003). Eotaxin-2 and eotaxin-3 are only distantly related to eotaxin-1 since they are only about 30% identical in sequence and are located in a different chromosomal position (Shinkai *et al.* 1999; Zimmermann *et al.* 2000b). The specific activity of all eotaxins is mediated by selective expression of the seven-transmembrane spanning, G-protein-coupled receptor CCR3, primarily expressed on eosinophils (Murphy 1994; Daugherty *et al.* 1996; Ponath *et al.*

1996). Notably, the eotaxin chemokines cooperate with IL-5 in the induction of tissue eosinophilia. IL-5 increases the pool of eotaxin-responsive cells and primes eosinophils to respond to CCR3 ligands (Zimmermann *et al.* 2003). Furthermore, when given exogenously, eotaxins cooperate with IL-5 to induce substantial production of IL-13 in the lung (Zimmermann *et al.* 2003). The finding that IL-4 and IL-13 are potent inducers of the eotaxin chemokines by a STAT6-dependent pathway provides an integrated mechanism to explain the eosinophilia associated with Th2 responses (Zimmermann *et al.* 2003). Recent studies have identified that eosinophil recruitment to the lung is dependent on STAT6 and a bone marrow-derived lung tissue resident non-T or B cell (Voehringer *et al.* 2004); in particular, eotaxin-2 production by airway macrophages likely accounts for this (Pope *et al.* 2005a,b). Of further interest, recently CCR3 has been shown to also deliver a powerful negative signal in eosinophils, depending on the ligand engaged. For example, pretreatment with the chemokine Mig inhibits eosinophil responses by a CCR3- and Rac2-dependent mechanism (Fulkerson *et al.* 2005).

Utilizing eotaxin-1 and eotaxin-2 single and double gene-deficient mice or neutralizing antibodies, both chemokines have been shown to have nonoverlapping roles in regulating the temporal and regional distribution of eosinophils in an allergic inflammatory site (Rothenberg *et al.* 1997; Gonzalo *et al.* 1998; Pope *et al.* 2005a). Utilizing a standard experimental asthma model induced by systemic sensitization with ovalbumin/alum followed by respiratory ovalbumin challenge, only a modest reduction in lung eosinophils was found in CCR3-deficient mice (Humbles *et al.* 2002). However, when the same CCR3-deficient mouse line was subjected to experimental asthma induction by epicutaneous ovalbumin sensitization, there was a marked deficiency of lung and BAL eosinophils (Ma *et al.* 2002). It was proposed that these apparently conflicting results may be related to the sensitization protocol (Ma *et al.* 2002), but the reason for this apparent discrepancy remains unclear. Notably, another CCR3-deficient mouse strain has recently been shown to have a profound reduction in eosinophil recruitment to the lung in the standard ovalbumin/alum systemic sensitization model (Pope *et al.* 2005b).

There is now substantial preclinical evidence supporting a role for the eotaxin chemokines in human allergic disease (Zimmermann *et al.* 2003). Experimental induction of cutaneous and pulmonary late-phase responses in humans has revealed that the eotaxin chemokines are produced by tissue-resident cells (e.g., respiratory epithe-

lial cells and skin fibroblasts) and allergen-induced infiltrative cells (e.g., macrophages and eosinophils). Following allergen challenge in the human lung, eotaxin-1 is induced early (6 hours) and correlates with early eosinophil recruitment; in contrast, eotaxin-2 correlates with eosinophil accumulation at 24 hours (Zimmermann *et al.* 2003). In another study, eotaxin-1 and eotaxin-2 mRNA were increased in patients with asthma compared with normal controls; however, there was no further increase following allergen challenge (Zimmermann *et al.* 2003). In contrast, eotaxin-3 mRNA was dramatically enhanced 24 hours after allergen challenge (Zimmermann *et al.* 2003). The chemoattractant activity of BAL fluid from patients with asthma is inhibited by antibodies against RANTES, MCP-3, MCP-4, and eotaxin-1 (Zimmermann *et al.* 2003). Further support for an important role of eotaxin-1 in human asthma is derived from analysis of a single nucleotide polymorphism (SNP) in the eotaxin-1 gene. A naturally occurring mutation encoding a change in the last amino acid in the signal peptide (alanine → threonine) results in less effective cellular secretion of eotaxin-1 *in vitro* and *in vivo* (Nakamura *et al.* 2001). Notably, this SNP is associated with reduced levels of circulating eotaxin-1 and eosinophils, and improved lung function (e.g., forced expiratory volume in 1 s or FEV₁) (Nakamura *et al.* 2001). Furthermore, an SNP in the eotaxin-3 gene is associated with atopy in a Korean population and eosinophilic esophagitis in a white population (Chae *et al.* 2005; Blanchard *et al.* 2006). Recently, the activity of eotaxin-1 and eotaxin-2 in humans has been investigated by injection of these chemokines into the skin of humans; both eotaxin-1 and eotaxin-2 induce an immediate wheal and flare response associated with mast cell degranulation and subsequent infiltrations by eosinophils, basophils, and neutrophils (Menzies-Gow *et al.* 2002). The infiltration by neutrophils is likely to be mediated indirectly by mast cell degranulation. These results provide substantial evidence that the biological activities attributed to eotaxins in animals are conserved in humans.

ROLE OF EOSINOPHILS IN DISEASE

Eosinophils are closely associated with infection by parasitic helminths, as production of Th2 cytokines, specifically IL-5, within cells in infected tissue promotes expansion of progenitor populations in the bone marrow, leading to blood and tissue eosinophilia (Pearce *et al.* 2004; Wynn *et al.* 2004; Jankovic *et al.* 2006). Although

in vitro studies suggest that eosinophils can destroy these organisms via secretion of cytotoxic proteins and reactive oxygen species, the results from infection studies carried out *in vivo* remain unclear and controversial.

Eosinophils are also closely associated with the pathogenesis of allergy, specifically in the respiratory tract, with the development of allergic asthma (Bochner & Busse 2005). Symptomatic wheezing and bronchoconstriction associated with asthma exacerbations can result from a superimposed respiratory virus infection, with common inciting agents including the paramyxovirus pathogen respiratory syncytial virus (hRSV) (Singh *et al.* 2007). At the same time, primary hRSV infection has intriguing associations with the development of childhood asthma (Everard 2006; Martin *et al.* 2006; Schaller *et al.* 2006). In connection with these observations, eosinophil recruitment and degranulation has also been observed in response to primary infection with hRSV, an observation that has been explored in human tissues, in culture systems, and in mouse models.

INFECTION

Eosinophils and parasitic helminth infection

Profound blood and tissue eosinophilia are among the hallmark features of parasitic helminth infection, observed in response to activation of CD4⁺ Th2 lymphocytes at specific stages of the parasite life cycle. As noted earlier, although it seems logical to conclude that eosinophils serve as a means of host defense, there are no "errors of nature" (i.e., human conditions or syndromes characterized by a unique eosinophil deficiency) that might provide direct insight into eosinophil function in helminth-related disease. The only eosinophil-specific condition is hereditary EPO deficiency (Romano *et al.* 1994), which is detected by laboratory analysis and which has not been related to an increased susceptibility to helminth infection in human studies. Interestingly, most of the human data available suggest a role for eosinophils in preventing reinfection (Hagan *et al.* 1985; Sturrock *et al.* 1996), a subject that might be addressed further in rodent infection models (Knopf *et al.* 1977). At the same time, controlled mouse model studies of primary infection have yielded results that are equivocal and, as such, no specific conclusions regarding the role of eosinophils in promoting host defense can be reached. There are several recent reviews that discuss these data in great depth, and provide significant insight into the ongoing controversy (Behm & Ovington 2000; Meeusen

& Balic 2000; Klion & Nutman 2004). The primary points are summarized here.

The initial paradigm, that eosinophils might provide host defense against parasitic helminths, came from studies in which activated human eosinophils in the presence of antibody and/or complement, as well as specific eosinophil secretory components alone (e.g., MBP, ECP, EPO), reduced the viability of various helminths *in vitro* (Butterworth & Franks 1975; Glauert & Butterworth 1977; Hamann *et al.* 1987). The availability of a monoclonal antibody directed against the eosinophilopoietic cytokine IL-5 permitted studies of the role of eosinophils in host defense against helminth infection to be performed *in vivo*. Although this reagent resulted in a large-scale reduction in circulating and tissue eosinophilia, there was no evidence for any change in the nature or extent of helminth infection in mouse model studies (Sher *et al.* 1990a; Herndon & Kayes 1992). Similar conclusions were reached in several studies performed in genetically altered IL-5 transgenic hyper-eosinophilic mice and in IL-5 and IL-5R α gene-deleted, eosinophil-deficient mice (Hokibara *et al.* 1997; Takamoto *et al.* 1997; Le Goff *et al.* 2000), as well as more recently in studies performed in the Δ dbl-GATA and TgPHIL eosinophil-ablated strains of mice (Swartz *et al.* 2006). However, among the notable exceptions, eosinophils did appear to play a role in reducing the parasite burden in several studies performed with nematode *Strongyloides* and *Angiostrongylus* species. Among these experiments, Korenaga *et al.* (1991) demonstrated increased recovery of *S. venezuelensis* worms from lungs of eosinophil-depleted mice treated with anti-IL-5 monoclonal antibody, and Sasaki *et al.* (1993) and Yoshida *et al.* (1996) demonstrated prolonged survival and increased recovery of *A. cantonensis* worms from anti-IL-5-treated and IL-5R α -deficient mice, respectively. Given the unique tissue migratory phase of *Strongyloides* and related nematode species, a role for eosinophils in host defense against specifically tissue-invading helminths has been suggested (Klion & Nutman 2004). A compilation of the reports documenting the results of mouse model studies that have addressed the role of eosinophils in host defense against helminth pathogens is shown in Table 12.3.

There are a number of publications that have focused on the role of eosinophils in promoting tissue pathology. A specific role for eosinophils in promoting pathology has been defined in mouse models of corneal inflammation characteristic onchocercal keratitis (Pearlman *et al.* 1998). However, in other models, such as those exploring

the pathogenesis of *Schistosoma mansoni* and *Nippostrongylus brasiliensis* infection in eosinophil-deficient mice, the characteristic liver and lung lesions, respectively, are eosinophil depleted, but pathology remains

Table 12.3 Eosinophils and host defense against helminth parasites: results from mouse model studies. (Adapted from Klion & Nutman 2004 with permission.)

Organism	Results
Cestodes	
<i>Mesocostoides corti</i>	–
Trematode	
<i>Fasciola</i> sp.	–
<i>Schistosoma</i> sp.	–
Nematodes	
<i>Angiostrongylus</i> sp.	+
<i>Heligmosomoides polygyrus</i>	–
<i>Brugia</i> sp.	+
<i>Nippostrongylus</i> sp.	±
<i>Onchocerca</i> sp.	–
<i>Strongyloides</i> sp.	+
<i>Toxocara</i> sp.	–
<i>Trichinella</i> sp.	±
<i>Trichuris</i> sp.	–

–, Eosinophils shown to play no role; +, eosinophils shown to provide host defense; ±, conflicting information in the literature.

otherwise unchanged (Coffman *et al.* 1989; Sher *et al.* 1990b; Swartz *et al.* 2006) (Fig. 12.8).

There are many reasons why it may be difficult to discern a role for eosinophils *in vivo* in the mouse experimental system. Among the possibilities, many of these experiments are performed with human pathogens that do not naturally infect rodent species, and thus there is no assurance that one is engaging innate host defense in an evolutionarily meaningful fashion. Related to this point, mouse eosinophils and human eosinophils are not necessarily functionally equivalent (Lee & Lee 2005). While EPO is highly conserved between mouse and human, the eosinophil ribonucleases and MBP are highly divergent. Likewise, mouse and human eosinophils display significant differences in morphology, surface protein expression, and propensity to degranulate in response to physiologic stimuli (Denzler *et al.* 2001; Clark *et al.* 2004; Lee & Lee 2005). The inability to observe eosinophils degranulating in response to infectious stimuli *in vivo* would appear to be a major factor hindering the identification of the role of this cell in host immunity against helminth infection. Different responses observed among various inbred strains of mice likewise add to the overall complexity (Dehlawi & Goyal 2003). Furthermore, it may be simply that we are asking the wrong questions. It remains possible that eosinophils do contribute to host defense, not

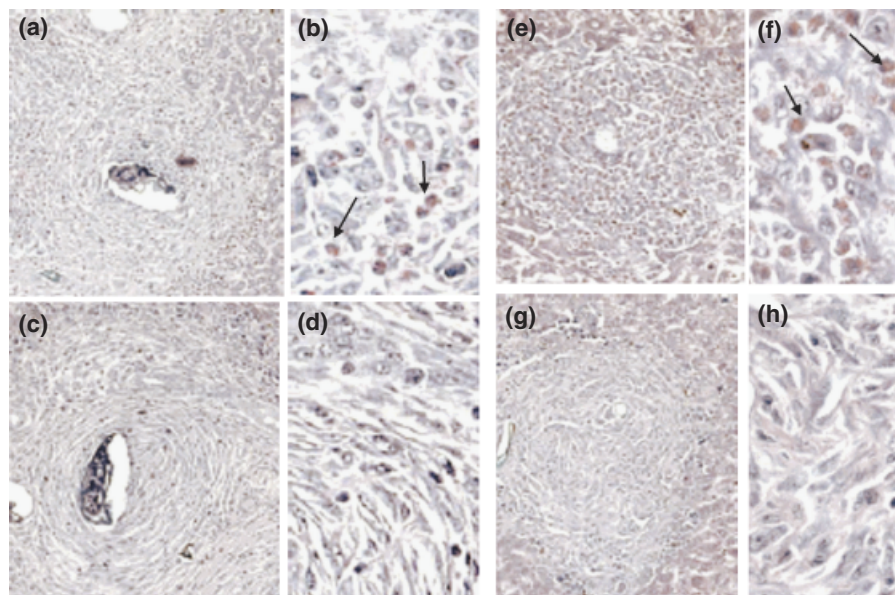


Fig. 12.8 Microscopic pathology of hepatic granulomas of *Schistosoma mansoni*-infected wild-type and eosinophil-ablated Δ dbl-GATA and TgPHIL mice. Giemsa-stained liver tissue sections featuring granulomas from *S. mansoni*-infected BALB/c (a, b), eosinophilablated Δ dbl-GATA (c, d), C57BL/6 (e, f), and eosinophil-ablated TgPHIL (g, h) mice, all at 12 weeks of infection. Arrows indicate examples of eosinophils. Original magnifications: $\times 10$ (a, b, e, f); $\times 40$ (c, d, g, h). (From Swartz *et al.* 2006 with permission.)

necessarily by reducing the number of pathogens or eliminating immediate pathologic responses, but instead in a more subtle fashion by contributing to tissue remodeling (De Jesus *et al.* 2004; Reiman *et al.* 2006), propensity for reinfection (Knopf *et al.* 1977; Sturrock *et al.* 1983), and/or other long-term immunomodulatory sequelae.

Eosinophils and respiratory syncytial virus infection

hRSV is single-stranded negative-sense RNA virus pathogen of the family Paramyxoviridae, subfamily Pneumovirinae that causes respiratory tract infection, primarily among infants and toddlers. The severity of infection can extend from mild upper respiratory symptoms to full-blown bronchiolitis and pneumonia, and may progress to acute respiratory distress syndrome and death, particularly among highly susceptible populations (reviewed in Tripp 2004; DeVincenzo 2005). There is no specific treatment for this infection other than primary support, as neither the antiviral agent ribavirin nor antiinflammatory glucocorticoids have proven impact on the course of disease (Randolph & Wang 1996). A safe and effective human vaccine is not available, although

humanized monoclonal antibody directed against the virus fusion (F) protein is approved for treatment of high-risk infants (Cardenas *et al.* 2005).

Several independent groups have detected eosinophils and/or their degranulation products in BAL washings taken from infants undergoing mechanical ventilation secondary to severe hRSV disease (Garofalo *et al.* 1992; Harrison *et al.* 1999; Dimova-Yaneva *et al.* 2004; Kim *et al.* 2006). The signals promoting eosinophil recruitment and degranulation have not been defined, although several potential eosinophil chemoattractants, including RANTES and MIP-1 α , have been detected in BAL from hRSV-infected infants (Harrison *et al.* 1999; Garofalo *et al.* 2001) (Fig. 12.9). Likewise uncertain is the role of eosinophils in primary hRSV infection, as there is no clear evidence from any human studies as to whether they promote host defense or serve to enhance immunopathology. Among these immunopathologies, there is a clear association between severe hRSV infection, particularly among the youngest infants, and the development of postinfection wheezing and asthma (Everard 2006; Martin *et al.* 2006; Schaller *et al.* 2006; Singh *et al.* 2007), the latter related, among other things, to age-dependent Th2 cytokine-mediated recruitment of

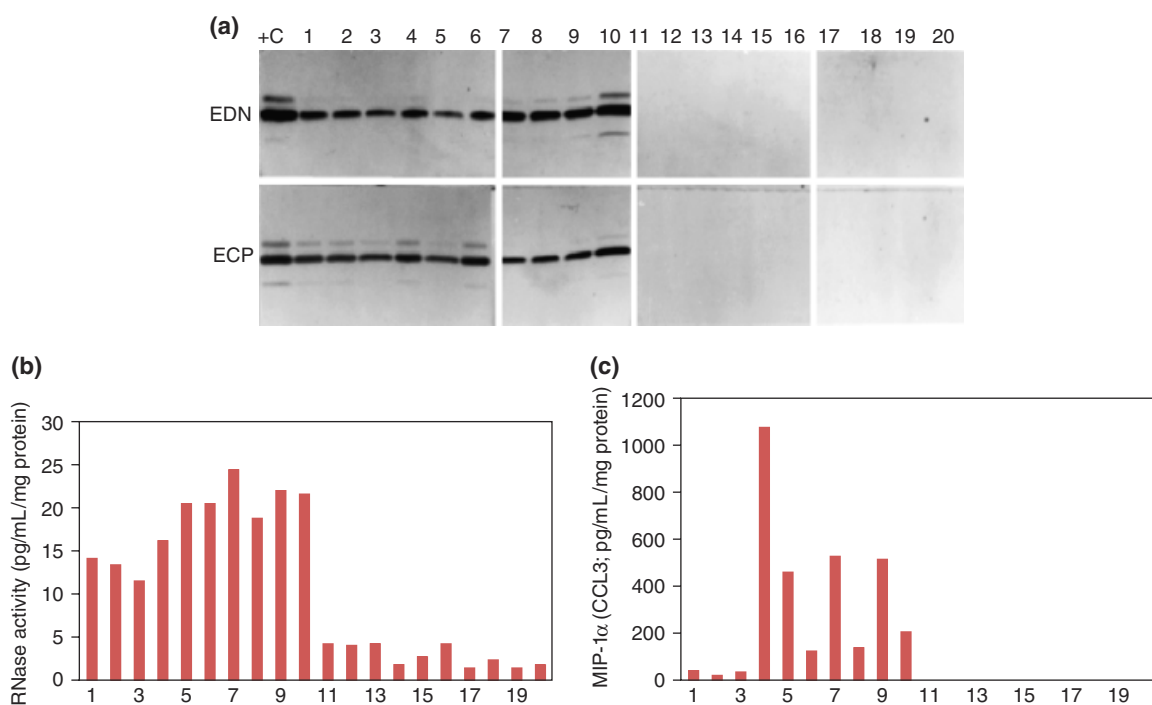


Fig. 12.9 Immunoreactive eosinophil granule proteins detected in lower airway secretions. (a) Western blots from lower airway secretions obtained from patients with RSV bronchiolitis (lanes 1–10) or unrelated diagnoses (lanes 11–20) were probed with polyclonal anti-EDN or anti-ECP antisera; C, control: human eosinophil lysate (< 1 μ g loaded). Detection of (b) ribonuclease activity (units/mL per mg protein) and (c) the proinflammatory chemokine MIP-1 α (pg/mL per mg protein) in the same samples. (Adapted from Harrison *et al.* 1999 with permission.)

proinflammatory eosinophils (Zhao *et al.* 2002; Kristjansson *et al.* 2005).

Specific observations made *in vitro* provide potential insight into the role of eosinophils in primary hRSV disease. In correlation with their presence in human BAL washings, hRSV-infected respiratory epithelial cells in culture synthesize both RANTES (CCL5) and MIP-1 α (CCL3) (Saito *et al.* 1997; Harrison *et al.* 1999; Miller *et al.* 2004). In support of a role in host defense, Domachowske *et al.* (1998) have shown that human eosinophils mediate a dose-dependent reduction in hRSV infectivity, an effect directly dependent on degranulation of its unique secretory ribonucleases. Likewise, Adamko *et al.* (2001) demonstrated that EPO inhibited replication of the related rodent RNA virus pathogen parainfluenza type I in a similar *in vitro* assay system.

The role of eosinophils in primary infection has also been addressed in the hRSV challenge model in mice. There are some differences between the human and mouse responses to the hRSV pathogen. As hRSV is a human pathogen, it undergoes little or no overt replication in mouse lung tissue and disease does not progress beyond a limited inflammatory state even in response to relatively large viral inocula. It is also important to recognize that most of the information relating to eosinophil recruitment in mice is based on studies of "enhanced disease," which is an IL-5-mediated allergic response to formalin-fixed virus and virion components (Piedra 2003; Openshaw & Tregoning 2005) and not directly related to primary hRSV infection *per se*. However, in a recent study, Phipps *et al.* (2007) demonstrated accelerated clearance of hRSV after primary challenge in IL-5 transgenic hypereosinophilic mice, an effect that is directly dependent on signaling through TLR7. Interestingly, eosinophil recruitment in the primary hRSV challenge model appears to be a function of age, with increasing numbers of eosinophils observed on infection of younger (neonatal) mice (Culley *et al.* 2002); neonatal challenge is also accompanied by enhanced recruitment of Th2 cells and progression to an allergen-responsive phenotype (You *et al.* 2006; Barends *et al.* 2004), similar to what has been observed in hRSV-infected human neonates.

The role of eosinophils in promoting host defense against natural rodent paramyxovirus pathogens has been explored in some detail. In a study directed toward understanding airway hyperresponsiveness, Adamko *et al.* (1999) identified an eosinophil-dependent reduction in titers of rodent parainfluenza I virus in lungs of mice subjected to ovalbumin sensitization and challenge.

Likewise, eosinophil recruitment has been observed as an early response to infection with pneumonia virus of mice (PVM), the cognate rodent pathogen most closely related to hRSV (Domachowske *et al.* 2000a; Easton *et al.* 2007). Eosinophil recruitment in response to PVM is not dependent on IL-5, but is blunted (along with recruitment of neutrophils) in mice devoid of the chemokine MIP-1 α or its receptor CCR1 (Domachowske *et al.* 2000b). Preliminary studies suggest that, similar to studies performed with hRSV (Phipps *et al.* 2007), higher titers of virus are detected in lung tissue of the eosinophil-ablated Δ dbl-GATA mice (Foster *et al.* 2007), although protection from the characteristic severe disease state is known to be related to factors other than absolute virus titer (Rosenberg *et al.* 2005).

In summary, eosinophils are recruited and degranulate in lung tissue in response to hRSV infection. Experiments performed *in vitro* and in several different mouse models suggest mechanisms underlying eosinophil recruitment, and provide evidence consistent with a role for these cells in promoting virus clearance.

ASTHMA

Asthma phenotypes

Asthma is a heterogeneous disease with several clinical subtypes and a wide spectrum, ranging from mild, episodic, wheezy breathlessness to chronic, intractable, corticosteroid-dependent chronic airway narrowing (Bel 2004). The classical IgE-associated allergic asthma phenotype starting in childhood is the most widely studied, not least because this form of the disease can be provoked in the clinical laboratory under controlled conditions by inhalation of allergen or allergen-derived T-cell peptides. In these patients, airway cells have a predominant Th2 cytokine profile (i.e., IL-4⁺, IL-5⁺, IL-9⁺ and IL-13⁺ mucosal cells). Some asthmatics have late-onset nonallergic (so-called "intrinsic") asthma in which sensitivity to allergens cannot be identified but in which airway eosinophilia and Th2 cells are also prominent (Humbert *et al.* 1999). The characteristic features of most asthma phenotypes, including allergic asthma, are airway inflammation, airway hyperresponsiveness (AHR), excessive airway mucus production due to goblet cell hyperplasia, and thickness of the airway wall. This airway thickness, often referred to as remodeling, is consequent to excessive repair processes following repeated airway injury and involves an increase in airway smooth muscle mass, deposition of collagen and other

matrix proteins, and new blood vessel formation (Bousquet *et al.* 1990).

Association with eosinophils

Increases in eosinophils in the tissues, blood, and bone marrow are a hallmark of most asthma phenotypes and, in general, elevated numbers correlate with disease severity (although "noneosinophilic/nonneutrophilic" asthma is characteristic of bacterial, viral, and pollutant triggers) (Douwes *et al.* 2002). This has led to the hypothesis that the eosinophil is the central effector cell responsible for ongoing airway inflammation. Thus, the cell has the potential to cause damage to the airway mucosa and associated nerves through the release of granule-associated basic proteins (which damage nerves and epithelial cells), lipid mediators (which cause bronchoconstriction and mucus hypersecretion), and reactive oxygen species (which generally injure mucosal cells).

The inflammatory milieu promotes the survival of eosinophils by the elaboration of agents that delay apoptosis. These include epithelial-derived GM-CSF and neurotrophins (e.g., NGF and brain-derived neurotrophic factor) (Hahn *et al.* 2006). Eosinophils are also highly sensitive to Fas-mediated apoptosis. In a mouse model of asthma, Fas-positive T cells were found to regulate the resolution of airway inflammation since Fas deficiency on T cells produced long-term allergic airways disease (Tong *et al.* 2006).

Blood eosinophils from patients with asthma have a number of phenotypic alterations, particularly in relation to their adhesive properties. Thus airway eosinophils recovered after antigen challenge have enhanced adhesion to VCAM-1 (CD106) and other ligands including albumin, ICAM-1 (CD54), fibrinogen, and vitronectin. These hyperadhesive properties seem to be mediated by upregulated and activated α M β 2 (CD11b/18) (Barthel *et al.* 2006). Asthmatic eosinophils also have increased expression of collagen receptors α 1 β 1 and α 2 β 1 integrins (Bazan-Socha *et al.* 2006).

More attention is now given to a possible role for the eosinophil in repair and remodeling processes since there is a well-documented association of tissue eosinophilia and eosinophil degranulation with certain fibrotic syndromes and the cell is the source of several fibrogenic and growth factors, including TGF- α , TGF- β , fibroblast growth factor (FGF)-2, vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-9, IL-1 β , IL-13, and IL-17 (see Fig. 12.6).

Eosinophils and animal models of asthma

Over the years animal models of asthma have often given conflicting results, especially those involving short-term sensitization (models of repeat allergen inhalation appear to be more reproducible). For example, two groups used experimental models of airway inflammation in mice genotargeted for complete and selective ablation of the eosinophil lineage. In one model eosinophils were targeted through transgenic expression of the diphtheria toxin A chain under control of the eosinophil peroxidase promoter (PHIL) (Lee *et al.* 2004). The authors of the study concluded that eosinophils were required for both AHR and mucus accumulation. Other workers have ablated the eosinophil lineage by deleting the high-affinity GATA-1 binding site on the palindromic GATA-1 promoter (Δ dbl) but found that these same features of experimental asthma were unaffected by eosinophil depletion, although the cell did appear to play a critical role in airway remodeling (Humbles *et al.* 2004). Such anomalies have previously been explained by differences in the strains of animals used (Shinagawa & Kojima 2003) and different experimental protocols.

Animal studies have demonstrated a role for eosinophils in airway remodeling. The Δ dbl-GATA animals were clearly protected from peribronchiolar collagen deposition and increases in airway smooth muscle (Humbles *et al.* 2004). Furthermore, in a chronic repetitive allergen challenge model, Cho *et al.* (2004) found that IL-5 gene deletion suppressed lung eosinophilia, peribronchial fibrosis, collagen III, collagen V and total lung collagen content in parallel. These changes were associated with decreased TGF- α 1 content of lung tissue, with evidence that eosinophils were the major source. Interestingly, epithelial cell expression of α v β 6, an integrin that activates latent TGF- β 1, was also suppressed. Peribronchial smooth muscle thickness and epithelial mucus expression were also reduced. Others have observed reduced airway eosinophilia, TGF- β production, and remodeling in IL-5 knockout animals, and increased airway fibrosis in IL-5 transgenic animals after repeated allergen inhalation (Tanaka *et al.* 2004). It was surprising therefore that the reduced subepithelial cell fibrosis observed in the Δ dbl-GATA line were apparently independent of TGF- β expression. However there are other potential fibrogenic pathway activators as shown diagrammatically in Fig. 12.10.

Remodeling in clinical asthma

The precise clinical significance of airway remodeling is debated. One view is that the thickness of the wall, the

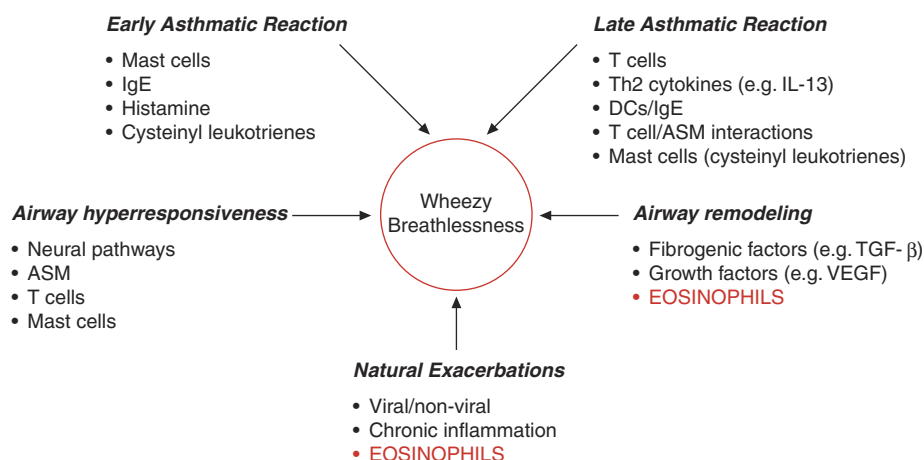


Fig. 12.10 Pathways to wheezy breathlessness in asthma. Airway narrowing, the cause of wheezy breathlessness, can result from several mechanisms, many of which overlap. Early and late reactions are clinical models. The early asthmatic reaction can be largely blocked by antihistamines and anti-cysteinyl leukotrienes and is largely mast cell/IgE-mediated. The late asthmatic reaction is partially dependent on the early response and is “blunted” by leukotriene receptor antagonists. The late-phase reaction also has a T-cell component and may involve antigen trapping and focusing by IgE-bound dendritic cells prior to presentation to, and activation of, Th2 cells. In airway hyperresponsiveness (AHR), nonspecific triggers such as smoke, dust, and fumes induce wheezing on a background of airway inflammation. The mechanisms underlying AHR include enhanced neural pathways, alterations in airway smooth muscle (ASM), and T cell- and mast cell-dependent pathways. Airway remodeling probably has a major eosinophil component as shown in both animals and human. Eosinophils are also prominent in natural exacerbations of the disease triggered by viral and nonviral agents. DC, dendritic cell; Th, T helper cell.

overall consequence of remodeling, leads to a decrease in baseline caliber (i.e., the radius of the airway lumen), resulting in a disproportionate increase in airway resistance, which in turn enhances AHR. Others point out that corticosteroids, the mainstay antiinflammatory treatment in the disease, reverse some but not all the features of remodeling. Nevertheless there is a hard core of asthmatics who are steroid resistant and airway remodeling is often quite marked in asthma deaths.

Studies in humans using anti-IL-5 antibodies also support a role for eosinophils in events surrounding deposition of certain matrix proteins within the reticular basement membrane (Kay *et al.* 2004). IL-5 is a key cytokine in eosinophil differentiation, maturation, recruitment, and activation at sites of inflammation. It stimulates the expansion and differentiation of eosinophil precursors and upregulates expression of its own specific receptor α chain during human eosinophil development. Anti-IL-5 has a good safety profile and anxieties regarding increased susceptibility to helminths and tumors appear, so far, unfounded. When asthmatics were given three infusions of anti-IL-5 (mepolizumab) this produced about a 90% reduction in blood and bronchial lavage eosinophils but only 55% reduction in bronchial mucosal eosinophils (Flood-Page *et al.* 2003b). However, even this modest effect produced significant reduction in tenascin, lumican, and procollagen III compared with placebo (Flood-Page *et al.* 2003c). There

was also a significant reduction in the numbers and percentage of tissue eosinophils expressing mRNA for TGF- β 1 as well as the concentration of TGF- β 1 in BAL fluid. Although there were no appreciable improvements in clinical outcomes, the study was not powered to detect changes in lung function or AHR. Nevertheless, the results do provide strong evidence that there is a causal relation between eosinophils and matrix deposition in the extracellular matrix. The clinical significance of these findings is unclear, especially as fibroblast accumulation and airway smooth muscle cell hypertrophy in proximal airways seem to be more selective determinants of severe persistent asthma than matrix deposition beneath the basement membrane (Benayoun *et al.* 2003).

A further complicating factor is the role of atopy. For example, airway eosinophilia and angiogenesis were observed in bronchial biopsies from atopic children without asthma (Barbato *et al.* 2006) and eosinophilic inflammation and increased AHR have been observed in adult patients with allergic rhinitis (Tatar *et al.* 2005). On the other hand, intense bronchial mucosal eosinophilia is a feature of nonatopic (“intrinsic”) asthma. One interpretation of these findings is that eosinophil-mediated damage precedes the development of overt asthma irrespective of the atopic status.

In order to provide definitive evidence that eosinophils are key cells in airway remodeling, more effective strategies are required to deplete tissue eosinophils. Even

in animal models of asthma there was residual tissue eosinophilia in the airways after anti-IL-5 administration (Foster *et al.* 2001). In fact depletion of both IL-5 and eotaxin are required to abolish tissue eosinophils and AHR in mice, suggesting that IL-5 blockade alone is insufficient. Combination therapy with, for example, anti-IL-5 and a CCR3 antagonist may be more useful than IL-5 blockade alone, since this would have the theoretical advantage of inhibiting both bone marrow maturation (mainly an IL-5 effect) and tissue accumulation (predominantly a CCR3-dependent effect). In a mouse model, ablation of eotaxin chemokines prevented antigen-induced pulmonary eosinophilia (Pope *et al.* 2005b) and antagonism of CCR3 reduced eosinophil numbers and this was accompanied by a diminution in asthma pathology (Weigmann *et al.* 2007).

Eosinophils are also known to localize to cholinergic nerves in a variety of inflammatory conditions including asthma. This effect appears to be the result of enhanced eotaxin production by neurons, possibly as a result of IL-4 and IL-13 upregulation (Fryer *et al.* 2006). These events result in damage of inhibitory M2 receptors by eosinophil MBP (Evans *et al.* 1997) and reduced catabolism of acetylcholine (Durcan *et al.* 2006).

Eosinophils and the late asthmatic reaction

When clinical asthma is provoked experimentally by inhalation of allergen, there are two general patterns of airway narrowing, termed the early asthmatic reaction (EAR) and late asthmatic reaction (LAR). The EAR (as measured by changes in FEV₁ as a test of airway narrowing) peaks within 15–30 min after allergen challenge and returns nearly to baseline by 1 hour. In this sense it is a “bronchospastic” reaction and involves the IgE-dependent release from mast cells of histamine and other mediators, including leukotrienes, prostaglandins, and tryptase. The LAR, on the other hand, is characterized by a delayed course and slow decrease in FEV₁ (which peaks between 3 and 9 hours), and tends to resolve by 24 hours. The mechanism(s) of the LAR is controversial, although there is good evidence to suggest that there is a significant T-cell component because isolated LAR (i.e., without the EAR component) can be provoked by inhalation of allergen-derived T-cell peptides (which do not cross-link IgE and cause mast cell activation) (Haselden *et al.* 1999). Furthermore, the immunosuppressant cyclosporin A blocks the late, but not the early, asthmatic reaction (Sihra *et al.* 1997). Thus the LAR may involve direct interaction between activated

T cells (presumed Th2 cells) and airway smooth muscle subsequent to IgE-dependent trapping and focusing by airway dendritic cells.

The role of the eosinophil in the LAR and in AHR remains uncertain. The original observation of Cockcroft *et al.* (1977) was that allergen inhalation increased AHR in dual asthmatic responders which was sustained for at least 7 days. The temporal association between increased inflammatory cell infiltration and increased AHR at 24 hours after allergen challenge in dual responders (Brusasco *et al.* 1990; Flood-Page *et al.* 2003b; Dorman *et al.* 2004a) has led to the suggestion of a causal relationship between the two. However, Kariyawasam *et al.* (2007) showed that allergen-induced increases in eosinophilic airway inflammation, while marked at 24 hours in dual asthmatic responders, were virtually resolved by 7 days. On the other hand, increases in AHR and expression of collagen markers of airway remodeling persisted. Other less direct evidence also supports the view that cellular inflammation, particularly eosinophil infiltration (Dorman *et al.* 2004b), does not necessarily directly relate to AHR (Djukanovic *et al.* 1990; Ollerenshaw & Woolcock 1992; Iredale *et al.* 1994; Crimi *et al.* 1998; Dorman *et al.* 2004b).

In an allergen-induced study by Gauvreau *et al.* (1999) on the cellular kinetics of cells in induced sputum, eosinophilia remained elevated 7 days after allergen inhalation challenge, albeit at levels considerably less than at 24 hours. This is in contrast to the finding of others who observed resolution of mucosal eosinophils to baseline levels by this time point (Kariyawasam *et al.* 2007). The reason for this difference between measures of airway luminal eosinophils and tissue cells is not clear but may reflect eosinophils that have been cleared from the submucosa but can still be detected in the sputum at a time point when tissue infiltration has resolved.

Attempts to deplete eosinophils selectively in humans have been largely unsuccessful and as such we have been unable to fully resolve the role of this cell in the asthma process. The first study in humans showed that a single infusion of anti-IL-5 produced a significant reduction in both blood and induced sputum eosinophils, but no appreciable changes in either the LAR or AHR (Leckie *et al.* 2000). However, a subsequent study (Flood-Page *et al.* 2003c) involving three infusions indicated that mepolizumab was unable to deplete tissue eosinophils, i.e., from the bronchial mucosa, making interpretation of the single infusion study problematic (O’Byrne *et al.* 2001; Flood-Page *et al.* 2002, 2003b; Leckie 2003). Nevertheless, further evidence against a role for eosinophils as causative of the late reaction comes from studies

in human skin in which it has been shown that the kinetics of eosinophil accumulation can be dissociated from the time course of the late-phase allergic reaction and that profound reduction of eosinophils (again by anti-IL-5) do not affect the magnitude of the allergen-induced cutaneous swelling and edema (Phipps *et al.* 2004a).

Eosinophils and airway hyperresponsiveness

There is no firm evidence that eosinophils or their products are directly causative in AHR in clinical asthma. The correlation between blood and tissue eosinophils and the degree of AHR is generally weak or nonexistent. Following a single infusion of anti-IL-5 there was no change in AHR, even when patients were followed up for several weeks, although this study may have been flawed for the reasons already stated above (Leckie *et al.* 2000). Further evidence casting doubt on a role for eosinophils in AHR comes from studies of eosinophilic bronchitis (Brightling *et al.* 2002, 2003). In this condition there is a similar distribution of eosinophils in the airways to that found in asthma, although there is no wheezy breathlessness or AHR (Brightling *et al.* 2003). The only histopathologic feature distinguishing asthma from eosinophilic bronchitis was mast cells associated with airway smooth muscle cells.

Eosinophils and natural exacerbations of asthma

Severe persistent asthma is characterized by viral- and nonviral-induced natural exacerbations on a background of chronic inflammation. An increase in blood or sputum eosinophils often predates deterioration in symptoms and lung function. In fact a management strategy directed at normalizing the sputum eosinophil count was more effective than traditional management strategy (based on lung function, assessment of symptoms, and use of rescue β_2 agonists) in reducing the number of asthma exacerbations (Green *et al.* 2002). It is interesting to speculate that eosinophil-derived fibrogenic/growth factors amplify airway remodeling and associated mucus production relatively rapidly and that this in turn leads to deterioration in symptoms. In a recent study it was shown that even a single allergen inhalation could induce acute airway remodeling in mild atopic asthmatics (Phipps *et al.* 2004b). Endobronchial mucosal biopsies obtained 24 hours after challenge showed significant increases in Hsp47, a chaperone of collagen synthesis as well as STAT6 and phospho-Smad2 as evidence of IL-4/IL-13 and TGF- β activated cells respectively. There were also increases in the thickness of

tenascin within the reticular basement membrane. Therefore ("eosinophilic") airway remodeling in asthma may partly result from repeated acute activation of the epithelial mesenchymal trophic unit by allergen exposure.

Conclusions

There are many areas of remodeling that require further investigation. The precise relationship between chronic inflammation and remodeling is still unclear. Some studies suggest that remodeling might even predate the first signs of inflammation and be an independent event that is amplified rather than caused by Th2 inflammation. The genetic predisposition to airway remodeling, including gene expression by resident cells from normal and diseased airways, is an important area for future research as is the precise effects of the various components of remodeling on airway function. For example, the consequence of subepithelial fibrosis to chronic airway obstruction is still unknown and the importance of angiogenesis, one of the few components of remodeling reversed by corticosteroids, in inflammation and edema is poorly understood.

Animal and human studies point to an important function of eosinophils in airway remodeling in asthma. The cell probably also plays a critical role in natural exacerbations of the disease. The significance of eosinophils in the LAR and in AHR, at least in the clinical situation, is far less certain. Studies on the role of the cell in AHR and mucus production, using eosinophil-lineage depletion in mice, has given diametrically opposite results, emphasizing the difficulties of animal models in mimicking the natural disease in humans.

Eosinophil-associated gastrointestinal diseases. Eosinophil accumulation in the gastrointestinal tract is a common feature of numerous gastrointestinal disorders, including classic IgE-mediated food allergy (Saavedra-Delgado & Metcalfe 1985; Moon & Kleinman 1995), eosinophilic gastroenteritis (Keshavarzian *et al.* 1985; Torpier *et al.* 1988), allergic colitis (Sherman & Cox 1982; Hill & Milla 1990; Odze *et al.* 1995), eosinophilic esophagitis (Furuta 1998; Rothenberg *et al.* 2001b; Fox *et al.* 2002), inflammatory bowel disease (IBD) (Sarin *et al.* 1978; Dvorak 1980; Walsh & Gaginella 1991), and gastroesophageal reflux disease (Winter *et al.* 1982; Brown *et al.* 1984; Liacouras *et al.* 1998). In IBD, eosinophils usually represent only a small percentage of the infiltrating leukocytes (Walsh & Gaginella 1991; Desreumaux *et al.* 1999), but their level has been proposed to be a negative prognostic indicator (Nishitani

et al. 1998; Desreumaux *et al.* 1999). Primary eosinophil-associated gastrointestinal diseases (EGIDs) such as eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, and eosinophilic colitis are defined as disorders that primarily affect the gastrointestinal tract with eosinophil-rich inflammation in the absence of known causes of eosinophilia (e.g., drug reactions, parasitic infections, malignancy). Patients with EGIDs suffer a variety of problems, including failure to thrive, abdominal pain, irritability, gastric dysmotility, vomiting, diarrhea, and dysphagia (Guajardo *et al.* 2002; Khan & Orenstein 2002). Evidence is accumulating to support the concept that EGIDs arise secondary to the interplay of genetic and environmental factors. Notably, a large percentage (~10%) of patients suffering from EGID have an immediate family member with EGID (Guajardo *et al.* 2002). Additionally, several lines of evidence support an allergic etiology: (i) about 75% of patients with EGID are atopic (Caldwell *et al.* 1975; Cello 1979; Scudamore *et al.* 1982; Furuta *et al.* 1995; Iacono *et al.* 1996; Sampson 1997; Walsh *et al.* 1999; Spergel *et al.* 2002); (ii) the severity of disease can sometimes be reversed by institution of an allergen-free diet (Kelly *et al.* 1995; Walsh *et al.* 1999; Spergel *et al.* 2002); and (iii) the common finding of mast cell degranulation in tissue specimens (Oyaizu *et al.* 1985; Bischoff 1996). Importantly, our recent models of EGID support a potential allergic etiology for these disorders (Rothenberg *et al.* 2001c). Interestingly, despite the common finding of food-specific IgE in patients with EGID, food-induced anaphylactic responses only occur in a minority of patients (Sampson 1999). Thus, EGIDs have properties that fall between pure IgE-mediated food allergy and cell-mediated hypersensitivity disorders (e.g., celiac disease) (Sampson 1999).

Hypereosinophilic syndromes

The term "hypereosinophilic syndrome" (HES) was introduced by Anderson and Hardy (1968) to designate patients with marked eosinophilia. They reported three patients, all males, between the ages of 34 and 47 who suffered from cardiopulmonary symptoms, fever, sweats, weight loss, and marked eosinophilia. Two of the patients died, and at autopsy their hearts were enlarged and showed mural thrombi. The treatment for HES is similar to that used for patients with chronic myelogenous leukemia, including prednisone, hydroxyurea (hydroxycarbamide), and interferon (IFN)- α . Chusid *et al.* (1975) formulated the diagnostic criteria for HES to include (i)

persistent eosinophilia, with an eosinophil cell count of at least $1.5 \times 10^9/L$ for a minimum of 6 months; (ii) lack of known causes for eosinophilia (e.g., parasitic or allergic triggers); and (iii) symptoms and signs of organ system involvement. Based on these diagnostic criteria, patients with EGID and blood eosinophil counts in excess of $1.5 \times 10^9/L$ meet the diagnostic criteria. However, patients with EGID generally do not have the high risk of life-threatening complications associated with classic HES (i.e., cardiomyopathy or central nervous system involvement). Notably, considerable heterogeneity among patients with HES has been recognized. For example, T-cell clones producing the characteristic Th2 cytokines IL-4 and IL-5 have been found in patients satisfying the diagnostic criteria for HES (Simon *et al.* 1999; Roufosse *et al.* 2003).

However, perhaps the most striking advance in our understanding of HES has come about following treatment of HES patients with the tyrosine kinase inhibitor imatinib mesylate (Schaller & Burkland 2001; Ault *et al.* 2002; Gleich *et al.* 2002; Cools *et al.* 2003; Cortes *et al.* 2003). Imatinib was introduced for the treatment of chronic myelogenous leukemia and has had a remarkable effect in that disease. Treatment of many HES patients with imatinib mesylate causes a dramatic reduction of peripheral blood and bone marrow eosinophils, suggesting that certain HES patients express a novel kinase sensitive to imatinib mesylate. Further investigation of the ability of imatinib mesylate to treat HES patients revealed the existence of an 800-kb deletion in chromosome 4 bringing together an upstream DNA sequence homologous to a yeast protein, referred to as FIP1, and designated as like FIP1 or FIP1-L1, and the gene for the cytoplasmic domain of the platelet-derived growth factor α (PDGFRA) receptor (Cools *et al.* 2003; Griffin *et al.* 2003). This fusion gene is transcribed and translated to yield a novel kinase referred to as FIP-L1-PDGFRA; FIP-L1-PDGFRA is exquisitely sensitive to imatinib *in vitro*, thus explaining the remarkable sensitivity of HES patients to this drug. The FIP-L1-PDGFRA fusion gene cooperates with IL-5 overexpression in a murine model of HES, suggesting that both pathogenic events cooperate in disease etiology (Yamada *et al.* 2006).

The patients responsive to imatinib are those most characteristic of "classic" HES, namely males between the ages of 20 and 50 who present clinically with marked peripheral blood eosinophilia. Recently, these patients have been shown to meet minor criteria for systemic mastocytosis, having elevated levels of serum mast cell tryptase and high numbers of dysplastic mast cells in the

bone marrow (Klion *et al.* 2003, 2004a). These patients go on to develop eosinophilic endomyocardial disease with embolization to peripheral organs including the extremities and the brain, and they strikingly resemble the patients originally designated by Hardy and Anderson. However, it appears that any disease that results in prolonged and marked eosinophilia can be associated with endomyocardial disease. For example, endomyocardial disease has occurred during the course of helminth infections and also in various malignancies associated with marked eosinophilia (Hussain *et al.* 1994; Yoshida *et al.* 1995; Andy *et al.* 1998). Thus patients with marked eosinophilia are at risk of developing cardiac disease regardless of the underlying etiology of the eosinophilia. Accordingly, routine surveillance of the cardiorespiratory system (e.g., echocardiography and plethysmography) in patients with EGID and peripheral blood eosinophilia is warranted. Based on these concerns, the diagnosis of HES in patients with EGID should always be considered, especially in those who develop extragastrointestinal manifestations (e.g., splenomegaly, or cutaneous, cardiac or respiratory systems). As such, additional diagnostic testing for HES should be considered including bone marrow analysis (searching for evidence of myelodysplasia), serum mast cell tryptase and vitamin B₁₂ levels (both moderately elevated in classic HES), and genetic analysis for the presence of the FIP1-L1-PDGFR fusion event (Klion *et al.* 2003).

ANTI-EOSINOPHIL THERAPEUTICS

Numerous drugs inhibit eosinophil production or eosinophil-derived products, including glucocorticoids, myelosuppressive drugs, leukotriene synthesis or receptor antagonists, tyrosine kinase inhibitors, IFN- α , and humanized anti-IL-5 antibodies. The etiology of the primary disease often specifies the best therapeutic strategy. For example, a subset of patients with HES have an 800-kb interstitial deletion on chromosome 4 (4q12) that results in the fusion of an unknown gene FIP1-L1 with PDGFR (Cools *et al.* 2003, 2004a,b). This fusion gene produces a constitutively active tyrosine kinase (PDGFR) that is exquisitely sensitive to the inhibitor imatinib mesylate, which is now approved for the treatment of HES (Gleevec). Although PDGFR is not normally active in hematopoietic cells, the activated kinase renders cells growth factor independent, perhaps by activating STAT5 signal transduction. Thus, eosinophilic patients with FIP1-L1-PDGFR-positive disease are now treated with Gleevec as first-line therapy (Gleich *et al.*

2002). In addition, a variety of other activated tyrosine kinases have just been associated with HES, including PDGFR- β , Janus kinase-2, and FGF receptor 1.

In most other individuals, glucocorticoids are the most effective agents for reducing eosinophilia (Rothenberg 1998). They suppress the transcription of a number of genes for inflammatory mediators, including the genes for IL-3, IL-4, IL-5, GM-CSF, and various chemokines including the eotaxins. Recently, the main action of glucocorticoids on eosinophil active cytokines has been shown to involve mRNA destabilization, thus reducing the half-life of cytokines such as eotaxins (Stellato *et al.* 1999). In addition, glucocorticoids inhibit the cytokine-dependent survival of eosinophils (Schleimer & Bochner 1994). Systemic or topical (inhaled or intranasal) glucocorticoid treatment typically causes a rapid reduction in eosinophils, but a few patients are glucocorticoid resistant and maintain eosinophilia despite high doses (Barnes & Adcock 1995). The mechanism of glucocorticoid resistance is unclear, but a reduced level of glucocorticoid receptors and alterations in transcription factor AP-1 (activator protein 1) appear to be at least partially responsible in some of them (Barnes & Adcock 1995).

Glucocorticoid-resistant patients sometimes require other therapy such as myelosuppressive drugs (hydroxyurea, vincristine) or IFN- α (Rothenberg 1998). IFN- α can be especially helpful because it inhibits eosinophil degranulation and effector function (Aldebert *et al.* 1996). Notably, patients with myeloproliferative variants of HES can often go into remission with IFN- α therapy. Cyclophilins (e.g., cyclosporin A) have also been used because they block the transcription of numerous eosinophil-active cytokines (e.g., IL-5, GM-CSF) (Rothenberg 1998). Recently, lidocaine has been shown to shorten eosinophil survival, and its effects mimic those of glucocorticoids and are noncytotoxic (Bankers-Fulbright *et al.* 1998). Indeed, an early clinical trial has shown that nebulized lidocaine is safe and effective in subjects with asthma (Hunt *et al.* 2004).

Drugs that interfere with eosinophil chemotactic signals include recently approved leukotriene antagonists and inhibitors. Inhibition of 5-lipoxygenase (e.g., zileuton) blocks the rate-limiting step in leukotriene synthesis and inhibits the generation of the eosinophil chemoattractant LTB₄ and the cysteinyl leukotrienes (Kane *et al.* 1996). Cysteinyl leukotriene receptor antagonists block the muscle contraction and increased vascular permeability mediated by leukocyte-derived leukotrienes (Gaddy *et al.* 1992). Some of the third-generation antihistamines inhibit the vacuolization (Snyman *et al.* 1992)

and accumulation (Redier *et al.* 1992) of eosinophils after allergen challenge and directly inhibit eosinophils *in vitro* (Rand *et al.* 1988; Snyman *et al.* 1992). Cromoglycate and nedocromil inhibit the effector function of eosinophils such as antibody-dependent cellular cytotoxicity (Rand *et al.* 1988).

The identification of molecules that specifically regulate eosinophil function and/or production offers new therapeutic strategies in the pipeline. Agents that interrupt eosinophil adhesion to the endothelium through the interaction of CD18/ICAM-1 (Wegner *et al.* 1990) or VLA-4/VCAM-1 may be useful (Kuijpers *et al.* 1993; Weg *et al.* 1993). Indeed, antibodies that block these pathways have recently been approved for other indications, but their anti-eosinophil activity has yet to be determined (von Andrian & Engelhardt 2003). Antibodies against IL-5, now humanized by two different pharmaceutical companies, are under active clinical investigation (Egan *et al.* 1995; Mauser *et al.* 1995) and look particularly promising for the treatment of HES (Garrett *et al.* 2004; Klion *et al.* 2004b, 2006) and eosinophilic esophagitis (Stein *et al.* 2006). While its utility for asthma may be limited due to redundant pathways, anti-IL-5 is particularly promising for HES. Numerous inhibitors of the eotaxin/CCR3 pathway, including small-molecule inhibitors of CCR3 and a human anti-eotaxin-1 antibody, are being developed (Zimmermann *et al.* 2003). Early results with a phase I trial of human antieotaxin-1 antibody in patients with allergic rhinitis have demonstrated the ability of this apparently safe drug to lower levels of eosinophils in nasal washes and nasal biopsies, and to improve nasal patency (Zimmermann *et al.* 2003). Antihuman IL-13 antibody is now in early clinical trials (Blanchard *et al.* 2005) and looks promising for lowering tissue eosinophil levels and improving features of asthma. Finally, a recently identified eosinophil surface molecule, Siglec-8, may offer a therapeutic opportunity (Nutku *et al.* 2003). Siglec-8 is a member of the sialic acid-binding lectin family and contains ITIMs (immunoreceptor tyrosine-based inhibitory motifs) that can induce efficient eosinophil apopto-

sis when engaged by anti-Siglec-8 cross-linking antibodies. It is interesting to note that Siglec-8, as well as CCR-3 and CRTH2, are coexpressed by other cells involved in Th2 responses including Th2 cells, mast cells, and basophils. Thus, agents that block these receptors may be particularly useful for allergic disorders.

CONCLUSIONS

Historically, eosinophils have been considered end-stage cells involved in host protection against parasites. However, numerous lines of evidence have now changed this perspective by showing that eosinophils are pleiotropic multifunctional leukocytes involved in initiation and propagation of diverse inflammatory responses, as well as modulators of adaptive immunity by directly activating T cells. As normal constituents of the mucosal immune system, particularly in the gastrointestinal tract, eosinophils are likely to have a physiologic function. Indeed, eosinophils have been implicated in innate immunity by being an early and possibly instrumental source of cytokines (e.g., IL-4) and have been shown to have a role in developmental processes such as mammary gland development. Analysis of recently generated genetically engineered eosinophil-deficient mice will soon answer critical questions concerning the true involvement of this cell type in a variety of processes. Breakthroughs in identifying key eosinophil regulatory cytokines such as IL-5 and the eotaxin subfamily of chemokines have uncovered mechanisms that selectively regulate eosinophil production and localization at baseline and during inflammatory responses. In particular, an integrated mechanism involving Th2 cell-derived IL-5 regulating eosinophil expansion in the bone marrow and blood and Th2 cell-derived IL-13 regulating eotaxin production now explains the means by which T cells regulate eosinophils. Based on these findings, it is predicted that targeted therapy against key eosinophil regulators (e.g., humanized anti-IL-5 and CCR3 antagonists) will likely transform medical management of eosinophilic patients.

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