Eosinophils: changing perspectives in health and disease

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Abstract | Eosinophils have been traditionally perceived as terminally differentiated cytotoxic effector cells. Recent studies have profoundly altered this simplistic view of eosinophils and their function. New insights into the molecular pathways that control the development, trafficking and degranulation of eosinophils have improved our understanding of the immunomodulatory functions of these cells and their roles in promoting homeostasis. Likewise, recent developments have generated a more sophisticated view of how eosinophils contribute to the pathogenesis of different diseases, including asthma and primary hypereosinophilic syndromes, and have also provided us with a more complete appreciation of the activities of these cells during parasitic infection.

Eosinophils were first described in 1879 by Paul Ehrlich, who noted their unusual capacity to be stained by acridine dyes. Interestingly, our appreciation of this unique property of eosinophils is clear and steadfast, but a comprehensive understanding of the function of these cells in health and disease remains elusive. Some basic characteristics of eosinophils are established and accepted. It is clear that eosinophils are granulocytes that develop in the bone marrow from pluripotent progenitors. They are released into the peripheral blood in a phenotypically mature state, and they are capable of being activated and recruited into tissues in response to appropriate stimuli, most notably the cytokine interleukin-5 (IL-5) and the eotaxin chemokines. Eosinophils spend only a brief time in the peripheral blood (they have a half-life of ~18 hrs) before they migrate to the thymus or gastrointestinal tract, where they reside under homeostatic conditions. In response to inflammatory stimuli, eosinophils develop from committed bone marrow progenitors, after which they exit the bone marrow, migrate into the blood and subsequently accumulate in peripheral tissues, where their survival is prolonged (reviewed in REFS 3–5).

However, much remains unclear. For example, the long-held belief that eosinophils promote immunity to helminths has been called into question by results from animal studies, some of which suggest that eosinophils may be serving to promote the needs and longevity of specific parasites. Likewise, eosinophils are clearly recruited to and activated in lung tissue as part of the pathophysiology of asthma, and most current evidence suggests that eosinophils contribute to airway dysfunction and tissue remodelling in this disorder. Evolution tells us that the ability to induce pathology cannot be a ‘raison d’etre’ for any existing cell lineage, and recent findings on the antimicrobial and antiviral activities of eosinophils suggest that the pathology that arises from dysregulated eosinophilia in the airways may be collateral damage related to host defence. Similarly, although there are now two unique eosinophil-deficient mouse strains, there are no known unique eosinophil-deficiency states in humans to help us to decipher the importance of these cells in vivo.

This Review examines the most recent advances in our understanding of the contributions of eosinophils to the maintenance of health, and how dysregulated eosinophil function promotes various disease states. These advances were made possible by reagents, systems and methods that target eosinophil function and by the first clinical trials using humanized monoclonal antibodies specific for IL-5 (TABLE 1). These tools have been invaluable for shaping our current views on eosinophil function and for generating new hypotheses for future examination.

The unique biology of the eosinophil

Relatively few mature eosinophils are found in the peripheral blood of healthy humans (less than 400 per mm3), but these cells can be readily distinguished from the more prevalent neutrophils by virtue of their bilobed nuclei and large specific granules (FIG. 1). Human eosinophil granules contain four major proteins: eosinophil peroxidase, major basic protein (MBP) and the
## Table 1 | Tools for the eosinophil biologist

| System                        | Specific reagent or model                                      | Characteristics                                                                 | Refs |
|-------------------------------|----------------------------------------------------------------|--------------------------------------------------------------------------------|------|
| **Cytological stains**        | Modified Giemsa                                                 | Stains the nucleus blue and granules bright red                                | 148  |
|                               | Sirius red                                                      | Stains the nucleus blue and granules red                                     | 149  |
|                               | Fast green and neutral red                                      | Stains the nucleus red and granules bright green                              | 150  |
| **Laboratory antibodies**     | Antibody specific for mouse IL-5Ra                              | Binds to the receptor for IL-5                                               | 151  |
|                               | Antibody specific for mouse SIGLEC-F                           | Binds to a sialic acid-binding immunoglobulin-like lectin expressed by eosinophils | 148  |
|                               | Antibody specific for mouse CCR3                               | Binds to the receptor for the eotaxins                                       | 152  |
| **Laboratory antibodies**     | Antibody specific for human EPX                                 | Monoclonal; does not cross-react with myeloperoxidase                      | 153  |
|                               | Antibody specific for mouse major basic protein                 | Binds to an eosinophil granule protein                                       | 154  |
| **ELISA assays**              | ELISA for human EDN                                             | Targets an eosinophil granule protein                                        | 155  |
|                               | ELISA for mouse EPX                                             | Targets an eosinophil granule protein                                        | 156  |
| **In vivo eosinophil**        | Treatment with TRFK5 antibody                                   | Rat monoclonal antibody that targets mouse IL-5                              | 157  |
| depletion**                   | Treatment with antibodies specific for mouse CCR3              | Rat antibodies that target mouse CCR3                                         | 158  |
|                               | Treatment with antibodies specific for mouse SIGLEC-F           | Depletes eosinophils by targeting a sialic acid-binding immunoglobulin-like lectin | 159  |
| **Methods for generating**    | Culture of human CD34<sup>+</sup> bone marrow cells with IL-5, IL-3 and GM-CSF | A cytokine-based method for generating eosinophils from human CD34<sup>+</sup> cells in vitro | 133  |
| eosinophils ex vivo**          | Culture of mouse bone marrow cells with SCF, FLT3L and IL-5     | A cytokine-based method for generating eosinophils from unselected mouse bone marrow progenitors in vitro | 160  |
| **Mouse strains**             | ΔdblGATA mice                                                   | Deletion of a palindromic GATA-binding site in the promoter of Gata1 results in the unique loss of cells of the eosinophil lineage | 10   |
| for manipulating eosinophils   | TgPHIL mice                                                     | The expression of diphtheria toxin A under the control of the Epx promoter results in the loss of eosinophil promyelocytes in the bone marrow | 11   |
| **ll5<sup>−/−</sup> mice**    | Il5 gene deletion; no eosinophilia in response to T<sub>H</sub>2 cell-inducing stimuli, although baseline eosinophil counts remain normal | 161  |
| **ll5ra<sup>−/−</sup> mice**  | Il5ra gene deletion; no eosinophilia in response to IL-5       | 162  |
| **Cd2−IL-5-transgenic mice**  | IL-5 overexpression is driven by the lymphocyte Cd2 promoter, resulting in systemic eosinophilia | 163  |
| **Cd3δ−IL-5-transgenic (NJ.1638) mice** | IL-5 overexpression is driven by the T cell Cd3δ promoter and enhancer region, resulting in systemic eosinophilia | 164  |
| **Ccl11<sup>−/−</sup> mice**  | Ccl11 gene deletion, resulting in diminished recruitment of eosinophils to the lungs and gastrointestinal tract | 165  |
| **Ccl24<sup>−/−</sup> mice**  | Ccl24 gene deletion; CCL24 is the dominant chemokine for allergen-associated eosinophil recruitment to the lungs | 166  |
| **Ccl11<sup>−/−</sup>Ccl24<sup>−/−</sup> mice** | Dual deletion results in profoundly diminished eosinophil recruitment in response to allergen sensitization and challenge | 167  |
| **IL-5/CCL24 double-transgenic mice** | Overexpression of IL-5 (as in NJ.1638 mice) and CCL24 (under the control of the lung-specific Cc10 promoter) elicits profound pulmonary eosinophilia and eosinophil degranulation in situ | 168  |
| **Ccr3<sup>−/−</sup> mice**   | Gene deletion of the receptor for eotaxins, resulting in diminished recruitment of eosinophils to tissues | 169  |
| **SiglecF<sup>−/−</sup> mice**| Exaggerated eosinophil responses and delayed resolution of lung eosinophilia in response to allergen challenge | 170  |
| **Humanized monoclonal antibodies** | IgG1-isotype antibodies specific for human IL-5 (mepolizumab and reslizumab (humanized TRFK5)) | Indirectly target eosinophils by depleting IL-5 | 96, 137, 171 |
| for clinical applications**   | IgG1-isotype antibody specific for human IL-5Ra (benralizumab) | Mediates the antibody-dependent cytotoxic destruction of eosinophils by targeting IL-5Ra | 96   |

CCL, CC-chemokine ligand; CCR3, CC-chemokine receptor 3; EDN, eosinophil-derived neurotoxin; ELISA, enzyme-linked immunosorbent assay; EPX, eosinophil peroxidase; FLT3L, FMS-like tyrosine kinase 3 ligand; GATA1, GATA-binding protein 1; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; IL-5Ra, IL-5 receptor subunit-a; SCF, stem cell factor; SIGLEC-F, sialic acid-binding immunoglobulin-like lectin F; T<sub>H</sub>2, T helper 2.
Eosinophils express surface receptors for ligands that support growth, adhesion, chemotaxis, degranulation and cell-to-cell interactions (Fig. 2). Many of the signalling pathways involved in these responses have been detailed in recent reviews. Among the main receptors that define the unique biology of the eosinophil are interleukin-5 receptor subunit α (IL-5Rα) and CC-chemokine receptor 3 (CCR3), as well as sialic acid-binding immunoglobulin-like lectin 8 (SIGLEC-8) in humans and SIGLEC-F (also known as SIGLEC-5) in mice. Pattern-recognition receptors (PRRs) are also likely to be important for eosinophil function, a subject that remains to be fully explored (Box 1).

Factors that promote eosinophilia

IL-5 has a central and profound role in all aspects of eosinophil development, activation and survival (Box 2). Likewise, CC-chemokine ligand 11 (CCL11; also known as eotaxin), which is a ligand for CCR3, promotes eosinophilia both cooperatively with IL-5 and via IL-5-independent mechanisms. Recently, several new factors that promote eosinophilic inflammation in vivo have been identified.

The epithelial cell-derived cytokines thymic stromal lymphopoietin (TSLP), IL-25 (also known as IL-17E) and IL-33 promote eosinophilia by inducing IL-5 production. TSLP is an IL-2 family cytokine that signals through a heterodimeric receptor that comprises the IL-7 receptor α-chain and a specific TSLP receptor β-chain. The TSLP receptor is expressed widely, by myeloid dendritic cells (DCs), CD4+ and CD8+ T cells, B cells, mast cells and airway epithelial cells. The TSLP receptor is also expressed by human eosinophils and modulates their survival and activation.

IL-25 is produced primarily by activated T helper 2 (T(H)2) cells and mast cells and induces the production of IL-5 from T(H)2 cells, as well as from the newly described populations of mouse innate lymphoid cells, which include nuocytes and natural killer helper cells. In this manner, IL-25 can amplify the development, recruitment and survival of eosinophils in allergic states. Abundant expression of both IL-25 and the IL-25 receptor was also detected in a recent study of bronchial and skin biopsies from allergic human subjects, and eosinophils themselves were identified as the primary source of IL-25 in patients with severe systemic vasculitis (Churg–Strauss syndrome).

IL-33 — which is a member of the IL-1 cytokine family — is expressed by epithelial cells, endothelial cells, fibroblasts and adipocytes, and is an endogenous danger signal known as an alarmin. IL-33 specifically modulates Th2-type pro-inflammatory signals following its release from necrotic cells. The IL-33 receptor ST2 (also known as IL-1RL1) is found primarily on T(H)2 cells, but IL-33-dependent responses from mouse nuocytes, natural helper cells and innate type 2 helper cells, and in human eosinophils themselves, have been described. Furthermore, an IL-33- and IL-25-responsive innate lymphoid cell population has recently been defined in humans. Although the biology of this cytokine has not been fully elucidated, IL-33 typically contributes to the synthesis and release of IL-5 from one or more of the aforementioned target cells, and thereby promotes systemic eosinophilia.

IL-23 is a member of the IL-12 family of cytokines that promotes the function of T(H)17 cells and also regulates allergic airway inflammation. Silencing the expression of IL-23 in mice that were sensitized and challenged with ovalbumin resulted in decreased recruitment of eosinophils to the lung tissue in association with diminished levels of IL-17 and IL-4 (Ref. 26). Accordingly, the overexpression of IL-23 was shown to augment antigen-stimulated eosinophil recruitment. However, another study found that IL-23 suppressed eosinophilia in a mouse model of fungal infection, a response that was IL-17 independent.

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High-mobility group protein B1 (HMGB1) is another example of an alamin that promotes eosinophilia. However, in contrast to IL-33, there is no evidence that eosinophil activation in response to HMGB1 involves IL-5. First identified as a nuclear protein and transcription factor, HMGB1 is expressed ubiquitously and mediates inflammatory responses via its receptors. The HMGB1 receptors that have been identified so far are receptor for advanced glycation end-products (RAGE), Toll-like receptor 2 (TLR2) and TLR4. Importantly, eosinophil mobilization and activation were observed in response to HMGB1 in tumour cell lysates. Further work is needed in this area, as a better appreciation of the way in which eosinophils are activated in response to HMGB1 and other, related damage-associated molecular patterns (DAMPs) may explain the eosinophil recruitment that is observed in the setting of tissue destruction associated with myalgias and myopathies.
**Box 1 | Receptors important for eosinophil function**

**Interleukin-5 receptor subunit-α**

The T helper 2 (T₂) cell-associated cytokine interleukin-5 (IL-5) has a unique and profound impact on nearly all aspects of eosinophil biology. Originally known as T cell replacing factor and murine B cell growth factor II, and later as eosinophil differentiation factor, IL-5 is produced by activated T₂ cells and, in smaller amounts, by mast cells, natural killer (NK) cells, natural killer T (NKT) cells and eosinophils themselves. In addition, several new sources of IL-5 have been identified in mouse models. These sources include Kit⁺ innate natural helper cells, nuocytes and IL-25- or IL-33-responsive innate IL-5-producing cells. IL-5 functions synergistically with the T₂-type cytokines IL-4 and IL-13, and with the eosinophil chemoattractants CC-chemokine ligand 11 (CCL11), CCL24 and CCL26 (also known as eotaxin, eotaxin 2 and eotaxin 3, respectively) to promote eosinophil activation and recruitment into tissues in acute inflammatory responses. As such, IL-5 receptor subunit-α (IL-5Rα) is the most prominent cytokine receptor associated with eosinophils.

In humans and mice, IL-5Rα is expressed by eosinophils and basophils. Mouse B1 cells also express IL-5Rα, and it functions to promote the proliferation and survival of these cells. The IL-5 receptor is heterodimeric; the α-subunit couples with a signalling β-subunit that is shared with the receptors for IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF). IL-5 receptor signalling promotes the development of eosinophils from committed progenitors, induces eosinophil activation and sustains eosinophil survival in peripheral blood and tissues.

Humanized IL-5-specific monoclonal antibodies (namely, mepolizumab and reslizumab) and a humanized IL-5Rα-specific monoclonal antibody (namely, benralizumab) are under exploration for the therapeutic management of dysregulated eosinophilia.

**Chemokine receptors**

CC-chemokine receptor 3 (CCR3) mediates eosinophil chemotaxis in response to the eotaxins, CCL11, CCL24 and CCL26 [REF 126]. CCR3 can also be activated by CCL5 (also known as RANTES), CCL7 (also known as MCP3), CCL8 (also known as MCP2) and CCL12 (also known as MCP5). Eosinophils also express CCR1 — which is the primary receptor for CCL3 (also known as MIPa1) and CCL5 — and the platelet-activating factor receptor.

**SIGLEC-8 and SIGLEC-F**

Sialic acid-binding immunoglobulin-like lectin 8 (SIGLEC-8) is a cell-surface immunoglobulin-like lectin that is expressed predominantly by human eosinophils. Mouse eosinophils express a functional parologue, SIGLEC-F [REF 127]. SIGLEC-8 and SIGLEC-F are members of a larger family of structurally related carbohydrate-binding proteins. Although the function of these proteins from the perspective of the eosinophil remains uncertain, antibodies specific for SIGLEC-8 or its recently identified carbohydrate ligand (6-sulpho sialyl Lewis X) promote selective eosinophil apoptosis. In particular, SIGLEC-8-specific antibodies exert this effect in physiologically relevant in vivo models. Thus, these SIGLEC proteins represent important targets for potential therapeutic ablation.

**Pattern-recognition receptors**

Several families of pattern-recognition receptors (PRRs) are expressed by eosinophils. Toll-like receptors (TLRs) are expressed by both human and mouse eosinophils, although at lower levels than by neutrophils and macrophages. TLR7 — which is localized in endosomes and detects single-stranded RNA — is by far the most prominent TLR expressed by eosinophils. It is not yet clear what exact role TLR7 has in promoting eosinophil function in vivo. However, priming eosinophils with IL-5 promotes responsiveness to the TLR7 ligand R837 and enhances the release of the pro-inflammatory cytokine IL-8 via unknown mechanisms. Activation of TLR7 regulates the adhesion, migration and chemotaxis responses of eosinophils and prolongs eosinophil survival.

**Cytolytic degranulation**

A mechanism through which eosinophils lyse, thereby releasing either free granule proteins or fully intact granules. This renders the cells non-viable. Intact granules released in this manner can respond to physiological secretagogues.

**Piecemeal degranulation**

A mechanism through which eosinophils (as well as basophils and mast cells) release specific mediators from cytoplasmic granules by transporting them to the cell surface in membrane-bound cytoplasmic vesicles. The eosinophils remain viable and fully responsive to subsequent stimuli.

**Secretagogues**

Substances that induce the secretion of another substance from a cell or storage granule.

**Eosinophil degranulation**

Degranulation － that is, the release of granule contents into the extracellular space － is a prominent eosinophil function. Previously, the release of secretory mediators was assumed to take place primarily through cytolytic degranulation, a mechanism through which a pathogenic assault (real or perceived) results in the complete emptying of the eosinophil’s arsenal of preformed cationic proteins. Interestingly, a careful analysis of electron micrographs of eosinophils degranulating in tissues suggested a more controlled process, which was given the name ‘piecemeal degranulation’ to reflect the fact that the eosinophil was able to release bits or pieces of its granule contents in response to a given stimulus, while remaining otherwise intact and apparently viable. Piecemeal degranulation is now accepted as the most commonly observed physiological form of eosinophil degranulation. Eosinophils undergoing piecemeal degranulation in response to cytokines, such as interferon-γ (IFNγ) and CCL11, develop cytoplasmic secretory vesicles, known as eosinophil sombrero vesicles (FIG. 2), and remain viable and fully responsive to subsequent stimuli.

A recent study has provided substantial insights into the molecular mechanism of piecemeal degranulation. Specifically, IL-4 released from CCL11-activated eosinophils first forms a complex with IL-4 receptor subunit-α (IL-4Ra) within the granule membrane, and IL-4Ra thereby chaperones IL-4 to the membrane vesicles before its release from the cell. Although receptor-mediated trafficking pathways have not yet been defined for other eosinophil mediators, this study provides an insight into the potential for exquisite molecular modulation of piecemeal degranulation.

Eosinophils also release intact granules, which are capable of storing and releasing mediators in response to physiological secretagogues in the cell-free state. Cell-free granules have been identified in tissues in association with eosinophil-associated disorders, although their functional significance and their ability to respond to activating stimuli in situ await further evaluation.
Eosinophils clearly promote the resolution of inflammation. To promote the resolution of inflammation, eosinophils use selectins and integrins to interact with endothelial cells, and they interact with epithelial cells at mucosal surfaces in a similar manner; these subjects have been reviewed extensively. Eosinophils also interact with and modulate the functions of other leukocytes (Fig. 3).

**Interactions with lymphocytes.** Eosinophils clearly respond to signals (such as IL-5) that are provided by T cells. Two recent studies indicate that T cells also respond to signals provided by eosinophils. Although not 'professional' antigen-presenting cells, eosinophils can express cell-surface components that are required for antigen presentation (such as MHC class II molecules and the co-stimulatory molecules CD80 and CD86). Indeed, eosinophils can process antigens and stimulate T cells in an antigen-specific manner, resulting in T cell proliferation and cytokine release. Furthermore, in experiments performed in both wild-type mice and transgenic mice that lack eosinophils (TgPHIL mice), eosinophils can augment allergic inflammation by regulating the production of Th2-type chemokine receptor (including CCL17 and CCL22), which promote the recruitment of Th2 cells, and also through their interactions with DCs. In addition, eosinophil release preformed cytokines (such as IL-4, IL-13 and IFNγ) that promote either Th2 or Th1 cell responses.

Eosinophils also promote humoral immune responses. Indeed, they are capable of priming B cells for the production of antigen-specific IgM. Most recently, the production of a proliferation-inducing ligand (APRIL) and IL-6 by eosinophils was shown to be crucial for the support of long-lived plasma cells in mouse bone marrow. Interestingly, activated eosinophils from the bone marrow of adjuvant-immunized mice were found to be even more effective at supporting plasma cell survival than those from adjuvant-naïve mice.

**Interactions with innate immune cells.** Alternatively activated macrophages have a pivotal role in recruiting eosinophils to the tissues through the release of YM1 (also known as CHI3L3), a chitinase-like selective eosinophil chemoattractant. Eosinophils likewise recruit alternatively activated macrophages to, and maintain their viability in, adipose tissue, promote the maturation of monocyte-derived DCs in vitro, and are required for the accumulation of myeloid DCs and the systemic production of Th2-type cytokines in mice with allergic airway disease. The eosinophil secretory mediator EDN promotes the activation and migration of DCs.

Eosinophils communicate extensively with tissue-resident mast cells. Eosinophils and mast cells are found in close proximity to one another under homeostatic conditions in the gut, and they also colocalize in the allergic lung and in the inflamed gut in patients with Crohn's disease. The bidirectional signalling that occurs between eosinophils and mast cells involves several immunomodulatory mediators. These include stem cell factor (also known as KIT ligand), granule proteins, cytokines (such as granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-3, IL-5 and tumour necrosis factor (TNF)), nerve growth factor and mast cell proteases. Actual physical coupling of eosinophils and mast cells has been observed both in vitro and in vivo, and this interaction prolongs eosinophil survival.
Eosinophil responses to pathogens and parasites

*Eosinophils and helminths: who wins?* The historic view that eosinophils promote host defence against helminths arose largely from histological images of eosinophils and parasites in tissue specimens and from *in vitro* studies that documented the antiparasitic activities of the eosinophil granule proteins MBP and ECP. With the development of reagents that block eosinophilia in mice (such as IL-5-specific antibodies) and of IL-5- or eosinophil-deficient mice, the picture has become more complex. For instance, the helminth Schistosoma mansoni, although not a natural mouse pathogen, can infect wild-type mice and can elicit a prolonged T helper 2 (Th2) cell response to allergen sensitization and challenge by producing CC-chemokine ligand 17 (CCL17) and CCL22 (REFS 40, 41). Eosinophils also prime B cells for antigen-specific IgM production and sustain long-lived plasma cells in mouse bone marrow via the production of a proliferation-inducing ligand (APRIL) and interleukin-6 (IL-6)44,45. Eosinophils that are stimulated by CpG DNA induce DC maturation46. Indeed, the eosinophil granule protein eosinophil-derived neurotoxin (EDN) promotes the maturation and activation of DCs50,51. Major basic protein (MBP) released from eosinophils activates neutrophils, causing them to release superoxide and IL-8 and increase their expression of the cell-surface integrin complement receptor 3 (CR3)52. Eosinophils also maintain alternatively activated macrophages in adipose tissue by producing IL-4 and IL-1353. The eosinophil granule proteins MBP, eosinophil cationic protein (ECP) and eosinophil peroxidase (EPX) activate mast cells, resulting in the release of histamine. Likewise, eosinophil-derived nerve growth factor (NGF) prolongs mast cell survival54.

*Angiostrongylus cantonensis* infection models, eosinophil depletion resulted in prolonged survival of tissue-based larval forms of the parasites55,56. Thus, the role of eosinophils in mouse models of helminth infection remains unclear and controversial.

The interaction of eosinophils and helminths during infection in human subjects has been examined using a genomics approach57. The 434G>C polymorphism in the gene encoding ECP results in substitution of the cationic amino acid arginine for the neutral amino acid threonine at position 97. The genotype 434CC — which encodes the more neutral and somewhat less cytotoxic form of ECP — is found commonly among Ugandans, who live in a region endemic for *S. mansoni*. By contrast, the 434CC genotype is quite rare in Sudan, where *S. mansoni* is not endemic. Although this result suggests that there is no selective advantage for those individuals whose eosinophils might provide stronger antischistosomal host defence, the authors of this study determined that individuals with the 434CC genotype...
developed substantially less liver fibrosis secondary to \textit{S. mansoni} infection. As such, the selective advantage may be for those individuals whose eosinophils promote less collateral tissue damage when faced with a similar pathogen burden. Similarly, cerebral malaria, a severe outcome of infection with \textit{Plasmodium falciparum}, is also associated with eosinophilia and elevated serum levels of ECP. The haplotype strongly associated with susceptibility to severe disease encodes arginine at position 97 and thus the more cationic form of ECP\textsuperscript{61}. The explanation of this finding awaits further clarification of the role of eosinophils in cerebral malaria.

The most recent developments in this field have exploited current concepts of eosinophils as immunomodulatory cells. In wild-type mice, infection with \textit{Trichinella spiralis} induces eosinophil recruitment to the infected tissues and the formation of nurse cells in skeletal muscle. In eosinophil-deficient ΔdblGATA and TgPHIL mice, \textit{T. spiralis} larvae do not survive, largely owing to the diminished recruitment of \textit{T}\textsubscript{h}2 cells and a concomitant increase in the activity of inducible nitric oxide synthase (iNOS) and the synthesis of nitric oxide in local macrophages\textsuperscript{62}. One interpretation of these results is that the parasites recruit eosinophils to support their own persistence and survival; another possibility is that eosinophils are recruited to maintain homeostatic balance by limiting the development of \textit{T}\textsubscript{h}1-type immune responses that lead to oxidative damage and tissue destruction. How the parasite elicits this response and whether this finding is unique to \textit{Trichinella} species are important subjects for future consideration. In addition, it will be interesting to address whether the mechanisms by which \textit{T. spiralis} recruits eosinophils to muscle tissue, the activation state of the eosinophils at this site and the mediators released \textit{in situ} are similar to those involved in eosinophilic inflammatory myopathies.

\textbf{Eosinophils and bacteria: pathogens, probiotics and the microbiome.} Early experiments carried out \textit{in vitro} documented the bactericidal properties of the cationic eosinophil granule proteins MBP and ECP\textsuperscript{62,63}. Subsequent studies exploring the mechanisms involved showed that ECP has a specific affinity for bacterial lipopolysaccharide and peptidoglycan and can agglutinate Gram-negative bacterial pathogens\textsuperscript{64}. More recently, \textit{in vivo} studies of the interaction of eosinophils with bacteria documented the catapult-like release of structures resembling neutrophil extracellular traps (NETs) from eosinophils, and this was associated with protection from the lethal sequelae of caecal ligation\textsuperscript{65}. In contrast to NETs, which are composed primarily of nuclear DNA and neutrophil-specific proteins, eosinophil NET-like structures are composed of mitochondrial DNA, MBP and ECP\textsuperscript{66}. Whether eosinophils and their secretory mediators have physiological bactericidal functions \textit{in vivo} requires further study. Although eosinophil-enriched IL-5-transgenic mice were protected from the lethal sequelae of \textit{Pseudomonas aeruginosa} infection\textsuperscript{67}, recent findings suggest that IL-5-mediated protection during bacterial sepsis might be mediated by cells other than eosinophils\textsuperscript{68}.

Recently, tremendous interest has developed regarding the immunomodulatory impact of probiotic or health-promoting bacteria. Although the mechanisms remain uncertain, oral administration of live probiotic \textit{Lactobacillus} or \textit{Bifidobacterium} species suppressed eosinophil recruitment in mouse models of allergic airway disease\textsuperscript{69,70}. However, the therapeutic impact of probiotics in human studies of allergic disease has been less impressive. Indeed, in a recent prospective study in which allergic children were provided with oral supplementation with \textit{Lactobacillus rhamnosus} GG or a placebo control, no significant differences were recorded in the number of asthma exacerbations per year, the number of days on medication, the peripheral blood eosinophil count or the serum ECP levels\textsuperscript{71}.

In parallel, the interactions between commensal bacteria and tissue-resident eosinophils in the intestine have been the subject of recent investigations. Mice raised under germ-free conditions exhibited exaggerated eosinophilia in a model of allergic airway inflammation; this phenotype was reversed when the gastrointestinal tract was colonized with normal microbiota\textsuperscript{72}. Likewise, a large prospective study involving over 400 healthy infants\textsuperscript{73} concluded that individuals with greater bacterial diversity in the gastrointestinal tract had a lower risk of developing allergic sensitization later in life.

\textit{Eosinophils and viruses.} Human respiratory viruses — such as influenza virus, parainfluenza virus, respiratory syncytial virus \textit{IL} \textsuperscript{69} and \textit{CD30} \textsuperscript{70,72} — are all associated with eosinophilia and elevated serum eosinophil counts. \textit{HIV} infection, typically in association with allergic inflammatory myopathies.

\textbf{Neutrophil extracellular traps (NETs).} Fibrous networks that are released into the extracellular environment by neutrophils. They are composed mainly of DNA, but also contain proteins from neutrophil granules. NETs act as a mesh that traps microorganisms and exposes them to neutrophil-derived effector molecules.
Asthma is a chronic inflammatory disease that is characterized by reversible airway obstruction and airway hyperreactivity in response to nonspecific spasmogenic stimuli. Eosinophils are a common feature of the inflammatory response that occurs in asthma, as they are recruited to the lungs and airways by cytokines that are released from activated T helper 2 cells and eosinophils that are released from activated T helper 2 cells and a range of chemokines, most notably those of the eotaxin family.

A role for eosinophils in promoting the pathogenesis of some forms of asthma is supported by a large body of literature, primarily from studies of acute and chronic allergen-challenged mouse models of allergic airway disease. Antigen sensitization and challenge, typically with ovalbumin or Aspergillus species, induces an allergic airway disease that replicates many of the hallmark features of allergic asthma, including increased numbers of cytokine-secreting T helper 2 cells and eosinophils in the airways, mucus hypersecretion and airway hyperreactivity. Chronic exposure to these antigens results in features of airway remodelling, including fibrosis and thickening of the basement membrane. Collectively, these studies suggest that targeting eosinophils themselves, eosinophil migration and/or eosinophilopoiesis should provide therapeutic benefit for the treatment of asthma.

These findings ultimately led to the development of two humanized IL-5-specific monoclonal antibodies, mepolizumab and reslizumab, which block the binding of IL-5 to IL-5Ra. In two of the earliest studies, mepolizumab was administered to patients with mild atopic asthma and to healthy volunteers. In response, eosinophil numbers in the bronchial mucosa decreased by 50%, an observation that correlated with reduced levels of the prominent pro-fibrotic eosinophil secretory cytokine, transforming growth factor-beta 1 (TGF-β1), and with diminished deposition of extracellular matrix proteins. Similarly, another study showed that mepolizumab suppressed eosinophil maturation in the bone marrow and resulted in fewer CD34+ IL-5Ra+ eosinophil progenitors in the lungs.

In initial clinical trials, small cohorts of patients with mild or moderate asthma were treated with mepolizumab or reslizumab, respectively.

These studies highlight the heterogeneous nature of asthma. The complexity, controversy and consensus of eosinophil accumulation in the airway wall and lumen is a prominent feature of asthma. However, the part played by eosinophils in promoting the cardinal features of this disorder has been the subject of recent controversy. Most available evidence from mouse models suggests that the activation of eosinophils contributes directly to the mucus production, bronchoconstriction and airway dysfunction and remodelling that are characteristic of allergic asthma. As such, eosinophils and molecules that regulate eosinophil development and recruitment are perceived as appropriate targets for therapeutic ablation.

One conflicting perspective emerged from studies of allergic airway disease in the two eosinophil-deficient mouse models (see Table 1). TgPHIL mice that were sensitized and challenged with an allergen responded as anticipated, with diminished mucus production and lower levels of airway hyperreactivity compared with wild-type mice.

By contrast, initial results from ΔdblGATA mice suggested that eosinophils had no role in promoting acute airway responses. These differences, once highly controversial, have since been attributed to variations in the mouse background strain.

At the same time, results from initial safety and efficacy trials of humanized IL-5-specific antibody therapy with eosinophil clearance and reduced eosinophil numbers in the blood and airways by cytokines that are released from activated T helper 2 cells and eosinophils that are released from activated T helper 2 cells and a range of chemokines, most notably those of the eotaxin family.

Another did not confirm this finding. Interestingly, the granule protein EDN has been shown to have HIV-inhibitory activity. However, the precise mechanisms by which eosinophils and their secretory mediators interact with viral pathogens remain to be elucidated.

**Eosinophils and disease**

There is extensive literature on eosinophil dysregulation associated with diseases such as asthma and eosinophilic oesophagitis. Although we know a substantial amount regarding how eosinophils develop and how they are recruited into various organs and tissues, there is a lack of understanding regarding the roles of eosinophils in eosinophil-associated diseases — even the relatively common ones. Targeting eosinophils therapeutically has revealed the complex and heterogeneous nature of eosinophil-associated diseases. We have selected the examples that follow to illustrate these principles; a more extensive list of diseases associated with eosinophilia is included in Supplementary information S1 (table) (see also REF. 85).

**Eosinophils and asthma.** Asthma is a chronic inflammatory disease that is characterized by reversible airway obstruction and airway hyperreactivity in response to nonspecific spasmogenic stimuli. Eosinophils are a common feature of the inflammatory response that occurs in asthma, as they are recruited to the lungs and airways by cytokines that are released from activated T helper 2 cells and eosinophils that are released from activated T helper 2 cells and a range of chemokines, most notably those of the eotaxin family.

**Box 3 | Eosinophils and asthma: complexity, controversy and consensus**

Eosinophil accumulation in the airway wall and lumen is a prominent feature of asthma. However, the part played by eosinophils in promoting the cardinal features of this disorder has been the subject of recent controversy. Most available evidence from mouse models suggests that the activation of eosinophils contributes directly to the mucus production, bronchoconstriction and airway dysfunction and remodelling that are characteristic of allergic asthma. As such, eosinophils and molecules that regulate eosinophil development and recruitment are perceived as appropriate targets for therapeutic ablation. 

One conflicting perspective emerged from studies of allergic airway disease in the two eosinophil-deficient mouse models (see Table 1). TgPHIL mice that were sensitized and challenged with an allergen responded as anticipated, with diminished mucus production and lower levels of airway hyperreactivity compared with wild-type mice. By contrast, initial results from ΔdblGATA mice suggested that eosinophils had no role in promoting acute airway responses. These differences, once highly controversial, have since been attributed to variations in the mouse background strain.

At the same time, results from the first safety and efficacy trials of humanized IL-5-specific monoclonal antibodies specific for interleukin-5 (IL-5) were published. The target populations for these trials were broadly defined, and included individuals with mild to moderate asthma. In these cohorts, the IL-5-specific antibodies were quite effective at removing eosinophils from the blood and the airways; however, no objective clinical benefits emerged. Although it was possible to conclude that eosinophils are unimportant in functional asthma pathogenesis, it was also evident that a large portion (up to 50%) of the eosinophils present in lung tissue were not removed and remained in the tissue both during and following the completion of the IL-5-specific antibody therapy.

The recognition of heterogeneity within the group of diseases currently classified as asthma has led to the introduction of the concept of disease endotypes, as well as of specific inflammatory phenotypes (namely, neutrophilic asthma, eosinophilic asthma, mixed granulocytic asthma and paucigranulocytic asthma). One of the most recent findings is that patients with poorly controlled, steroid-resistant eosinophilic asthma respond to IL-5-specific monoclonal antibody therapy with eosinophil clearance and marked improvements in important objective measures of disease.
as the cytotoxic IL-5Ra-specific monoclonal antibody benralizumab, or indirectly, such as the IL-13-specific monoclonal antibody lebrikizumab, may further enhance therapeutic outcomes.

Eosinophilic oesophagitis. Eosinophils are normally found in the gastrointestinal tract, notably in the caecum, but not in the oesophagus. First described by Landres and colleagues in 1978, eosinophilic oesophagitis is the most common of the eosinophil-associated gastrointestinal diseases. In 2007, an international consortium — the First International Gastrointestinal Eosinophil Research Symposium (FIGERS) — published consensus guidelines for diagnosis, which were revised in 2011. These criteria include: clinical evidence of oesophageal dysfunction (including dysphagia, abdominal pain and/or food bolus impaction); 2–4 biopsy samples from the proximal and distal oesophagus with ≥15 eosinophils per field at ×400 magnification; and no response to 6–8 weeks of high-dose proton-pump inhibitor therapy, ruling out gastro-oesophageal reflux disease. As we focus here on eosinophil-mediated mechanisms, we refer readers to a recent review on the complete natural history of eosinophilic oesophagitis.

All evidence points to dysregulated eosinophilia as being central to the pathophysiology of eosinophilic oesophagitis. The aetiology appears to be dependent on the T(H)2-type cytokines IL-5 and IL-13. Patients often report concurrent allergic responses to food and airborne allergens, along with a family history of allergy, and there is an unexplained male predominance. Although absolute eosinophil numbers in biopsy samples at any given time may or may not correlate directly with disease severity, evidence of eosinophil activation — including the presence of extracellular granules and degranulated cationic proteins (such as MBP) — is prominent in tissue biopsy samples. The eosinophil chemoattractant CCL26 (also known as eotaxin 3) is a prominent biomarker of eosinophilic oesophagitis. Indeed, CCL26 is highly upregulated in diseased tissues and also in peripheral blood cells in patients with this disorder. A single-nucleotide polymorphism (2,496T>G) in the 3’ untranslated region of the gene encoding CCL26 has been associated with increased susceptibility to eosinophilic oesophagitis, although the mechanisms involved are not yet known. Susceptibility to eosinophilic oesophagitis has also been correlated with polymorphisms in the gene encoding TSLP.

There are several mouse models of eosinophilic oesophagitis. Some of these models use oral or intranasal delivery of allergens to elicit tissue pathology, and others promote eosinophil recruitment to the oesophagus via the overexpression of IL-5 or IL-13. Among these models, one uses repeated intranasal delivery of fungal or insect aeroallergens, which induces the expression of T(H)2-type cytokines and the eotaxin family member CCL11 (mice do not express CCL26), resulting in eosinophil recruitment to the oesophagus. Another mouse model involves systemic sensitization with ovalbumin in aluminium hydroxide adjuvant followed by repeated intra-oesophageal challenge, which induces eosinophil recruitment associated with angiogenesis, basal zone hyperplasia and tissue fibrosis. Interestingly, although the administration of eosinophil-depleting SIGLEC-F-specific antibodies to these mice inhibits eosinophil recruitment and the associated tissue remodelling, in another investigation, in which oesophageal remodelling was driven by lung-specific expression IL-13, no role for eosinophils was observed. Similarly, ablation of CD4+ T cells — which presumably leads to a reduction in the levels of T(H)2-type cytokines — has only a limited impact on the recruitment of eosinophils to the oesophagus after chronic administration of Aspergillus species antigens. Among the issues to be addressed in future studies is the role of eosinophil degranulation into the oesophageal tissue in these mouse models. Furthermore, mouse models that incorporate relevant clinical symptoms, such as failure to thrive, would certainly be of significant value.

Current therapies for patients with eosinophilic oesophagitis include the introduction of an elemental diet and treatment with steroids, which target the global inflammatory response and have an impact on eosinophil-derived cytokines. Therapies that specifically target eosinophils are also being tested. For example, a randomized placebo-controlled double-blind trial in which adults with eosinophilic oesophagitis were treated with a humanized IL-5-specific monoclonal antibody (mepolizumab) resulted in a reduction in oesophageal inflammation and the reversal of tissue remodelling, but only minimal relief of symptoms. Similar results were obtained in a prospective study in children, with clinical improvement observed in both experimental and placebo groups. Interestingly, mepolizumab did not deplete eosinophils found in the duodenal mucosa of these patients. However, the aforementioned studies suggest that this disorder may be primarily regulated by CCL26. As with asthma, the stratification of patients into subgroups that respond to specific therapies may ultimately improve clinical outcomes.

Eosinophilic myopathies. These conditions are among the most rare and poorly characterized of the eosinophil-related disorders, and include eosinophilic fasciitis (also known as Shulman’s syndrome), toxic oil syndrome and eosinophilia–myalgia syndrome (BOX 4). Although eosinophils are associated with these conditions, it is not clear how they are recruited to the affected tissue or what their contributions are to the pathology observed.

Eosinophilic myositis is a relatively rare condition in which the infiltration of muscle tissue by eosinophils is observed, sometimes in association with peripheral blood and bone marrow eosinophilia. The disease can result from helminth infection, or it can be toxin induced or idiopathic in nature. Recently, specific mutations in the gene encoding calpain 3 were identified in association with idiopathic eosinophilic myositis. Calpain 3 is a muscle-specific neutral cysteine protease that interacts with intracellular myofibrillar proteins and has a role in sarcomere adaptation. However, there is no direct or obvious relationship between the actions of this enzyme and eosinophils or
Eosinophilia–myalgia syndrome. Eosinophilia–myalgia syndrome (EMS) is a multisystem disorder that was first formally documented in a 1989 report of three cases in which eosinophilia and myalgias were connected to the ingestion of L-tryptophan dietary supplements. Symptoms included severe muscle pain accompanied by profound peripheral eosinophilia. By 1990, more than 1,000 cases had been identified. The US Centers for Disease Control and Prevention defined EMS by three criteria: peripheral eosinophilia of ≥1,500 eosinophils per mm$^3$ in peripheral blood in the absence of any known aetiology. Although these disorders were recognized early on as clinically heterogeneous, recent studies have revealed the molecular basis for a few of the distinct phenotypes. The identification of myeloproliferative hypereosinophilic syndrome (MHES) emerged from the dramatic therapeutic responses observed in a subset of patients with hypereosinophilic syndrome following empirical treatment with imatinib, a tyrosine kinase inhibitor first developed for the treatment of chronic myeloid leukaemia. This clinical observation led to the detection of a deletion in chromosome 4 that results in the fusion of the genes encoding pre-mRNA 3’-end-processing factor FIP1 (FIP1L1) and platelet-derived growth factor receptor-α (PDGFRα). This leads to the production of a FIP1L1–PDGFRα fusion protein that constitutively activates proliferation and survival pathways, resulting in the clonal proliferation of eosinophils, elevated serum levels of tryptase and vitamin B12 (also known as cobalamin), severe peripheral eosinophilia and end-organ damage, the most severe form of which is endomyocardial fibrosis. Other fusion kinases have also been identified in individuals with MHEs; other individuals display clinical symptoms consistent with MHEs but without a clear molecular diagnosis. Thus far, all of the PDGFRα- or PDGFRβ-derived mutant fusion proteins that have been identified in humans have been associated with eosinophilia, for reasons that remain obscure.

The constitutive cellular activation and proliferation promoted by the FIP1L1–PDGFRα fusion protein has been explored in cell-culture models. For example, Ba/F3 immortalized mouse pro-B cells require the cytokine IL-3 for survival and proliferation in culture, but stable expression of the FIP1L1–PDGFRα fusion gene activates intracellular signalling pathways and eliminates the requirement for this cytokine. Likewise, imatinib inhibits the growth of the human eosinophil leukaemia EoL-1 cell line, which expresses the FIP1L1–PDGFRα fusion protein. Most intriguingly, the uncontrolled activity of the fusion protein lies within the PDGFRα component, as the fusion eliminates an inhibitory juxtamembrane region encoded by exon 12 of the PDGFRα gene, resulting in constitutive signalling by PDGFRα in the absence of its ligand.

In contrast to the myeloproliferative variants, eosinophilia in lymphocytic-variant hypereosinophilic syndrome (LHES) results from aberrantly activated T cell clones that constitutively produce eosinophilopoietic cytokines, including IL-5. The resulting eosinophilia is thus reactive. The aberrant T cell clones (which typically have a CD3+CD4+ phenotype) are also associated with elevated serum levels of IgE and CCL17, and elicit predominantly skin manifestations, including pruritus, eczema, erythroderma, urticaria and angio-oedema. Individuals with this diagnosis respond to treatment with steroids, with cytotoxic agents (such as hydroxyurea) and with mepolizumab, which reduced the requirement for corticosteroids in clinical studies.

These two defined variants of hypereosinophilic syndrome currently represent a minority of cases. Indeed, a recent study showed that FIP1L1–PDGFRα fusions were associated with only 11% of cases of hypereosinophilic syndrome, and LHES accounted for only 17% of cases. The classification of hypereosinophilic syndrome is currently a work in progress, and attempts are being made to balance the clinical diagnosis with the predicted response to therapy.

In an initial mouse model, bone marrow transplantation using haematopoietic progenitors that had been retrovirally transduced with FIP1L1–PDGFRα resulted in myeloproliferative disease. Another group created a model that combines features of both myeloproliferative and lymphocytic-variant disease by transducing...
Eosinophils: changing perspectives

The field of eosinophil research is one of changing perspectives and emerging new directions. Eosinophils are clearly capable of more sophisticated immune functions than previously thought, as shown by their nuanced degradation responses to distinct stimuli and their complex interactions with other leukocytes and pathogens. Both successful and unsuccessful attempts to target eosinophils have yielded remarkable insights into disease pathogenesis. Asthma and hypereosinophilic syndromes are now understood to be complex heterogeneous disorders that require tailored therapeutic strategies. Assessing the role of endogenous and exogenous PRR ligands in eosinophil responses and clarifying the relationship between eosinophil degradation and tissue remodelling will be important goals for future research. A better understanding of these and other aspects of eosinophil biology will aid the development of new therapeutic strategies for diseases characterized by eosinophil dysregulation.

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