In 1879, German scientist Paul Ehrlich identified eosinophils in the blood of several different mammals, including dogs, rabbits and humans, for the first time. Ehrlich found unique blood cells that consisted of bright red "alpha-granules" using the acidic bromide-containing dye eosin and named them "eosinophilic granulocytes". Eosinophils are now known as leukocytes containing large amounts of granules that include various types of toxic molecules accompanied by the expression of many types of cytokine receptors and chemokine receptors. IL-5 is a key cytokine for eosinophils, as it is essential for the development, survival and proliferation of eosinophils in vivo. When mucosal tissues are injured by parasitic infection, eosinophils release abundant cytokines, chemokines, lipid mediators and Charcot-Leyden crystals (CLCs) in the local foci to protect the host against helminth infection. Recent studies have also revealed that eosinophils play important roles in tissue homeostasis and repair. However, eosinophils are also involved in shaping the pathogenesis of allergic diseases, including fibrotic responses in asthmatics.

In this review, first, we will summarize the molecular mechanisms underlying the development of eosinophils in vivo. Second, we will introduce the pathogenic roles of eosinophilic ETosis (EETosis) and the various functions of granules, including CLCs, during eosinophilic inflammation. Finally, we will discuss the double-edged roles of eosinophils in tissue repair and type 2 immune inflammation.

Development of eosinophils expressing various types of functional cell surface molecules

In the human body, eosinophils are generated from CD34+ stem cells in the bone marrow. CD34+ stem cells proceed with development though granulocyte/macrophage progenitor (GMP) in mice and common myeloid progenitor (CMP) in humans. Distinct from other immune cells, such as neutrophils, monocytes, macrophages and lymphocytes, the activation of several transcription factors, such as GATA-1 (a zinc finger family member), PU.1 (an ETS family member) and C/EBP members (CCAAT/enhancer-binding protein family), is needed for the commitment of the eosinophil lineage-progenitor cells. Among these transcription factors, GATA-1 is the key molecule for eosinophil lineage specification because
Eosinophils are deficient in mice with a deletion of the high-affinity GATA-binding site in the GATA-1 promoter region. Another transcription factor, X-box binding protein 1 (XBP1), and its upstream activator inositol-requiring enzyme 1α (IRE1α) modulate the development of eosinophils but not that of other granulocytes, basophils and neutrophils. In contrast, transcription factors, such as friend of GATA protein 1 (FOG1), inhibitor of DNA binding 1 (Id1) and Kruppel-like factor 5 (Klf5), inhibit the development of eosinophils.

Eosinophils spend about eight days in the bone marrow during the process of maturation before entering blood vessels. During this maturation process in the bone marrow, the IL-5 receptor (IL-5R) expresses on eosinophil-restricted precursor (EoP). IL-5R is a heterodimer that consists of an IL-5Rα subunit and a βc subunit. Interestingly, IL-5 specifically binds to IL-5Rα and activates Janus kinase 2 (Jak2). The βc subunit is also associated with IL-3Rα and granulocyte-macrophage colony-stimulating factor receptor α (GM-CSFRα). IL-5, IL-3 and GM-CSF cooperate to promote the proliferation, development and activation of eosinophils.

Eosinophils are a relatively minor population among the circulating leukocytes. The ratio of circulating eosinophils is less than 5% in total leukocytes (<400/mm³, in case of healthy adults). At 8–12 h after entering circulation, eosinophils reach peripheral organs. Most eosinophils reside in various organs, such as the lung, liver, kidney, skin, heart, spleen, lymph nodes, thymus, adipose tissue, mammary glands, digestive tract and uterus. Eosinophils remain in those peripheral organs and tissues for 7–14 days and exert their function as tissue-resident cells in the tissue, as we discuss in the following section. Under steady-state conditions, <5% of lymphocytes in the lung are composed of eosinophils; however, eosinophils dramatically proliferate under inflammatory conditions, such as allergic diseases and helminth infections, via various stimulations.

Eosinophils express a variety of surface receptors associated with growth, adhesion, chemotaxis, degranulation, cell-to-cell interactions and pattern recognition (Fig. 1), including CC-chemokine receptor 3 (CCR3), sialic acid-binding immunoglobulin-like lesson 8 (Siglec-8, mouse parologue; Siglec-F), IL-5 receptor subunit α (IL-5Rα) and IFNγ receptor α-chain (IFNγR1). Importantly, eosinophils have the function of antigen presentation to bacteria, virus and helminths.

Eosinophils express MHC class II, together with CD80 and CD86, which are co-stimulation factors for T cell activation. Eosinophils can also present antigens to CD4+ T cells.

Muc4 and Muc5b, which are glycoproteins and mucins, have been identified as ligands of Siglec-F. Muc4 and Muc5b are expressed in the tracheal epithelial cells under steady-state conditions, and airway inflammation causes the enhanced expression of these molecules. In vitro, Muc4 and Muc5b bind to eosinophils and induce cell death. However, in vivo, Muc5b deficiency leads to exacerbated eosinophilic inflammation. These data indicate that Siglec-F is involved in the longevity of eosinophils.

**Eosinophil extracellular trap cell death (EETosis) exacerbaes eosinophilic inflammation**

Activated eosinophils die in a specific way during inflammation. Eosinophil cytolysis with lytic degranulation represents a specific process of cell death of activated eosinophils. Eosinophil cytolysis is known as extracellular trap cell death (EETosis), which differs from the processes of accidental necrosis or apoptosis. Eosinophil EETosis (EETois) is a reactive oxygen species (ROS)-dependent pathway, in which nicotinamide adenine dinucleotide phosphate (NADPH) oxidase induces nuclear envelope and plasma membrane disintegration together with the formation of extracellular traps. Eosinophil extracellular trap products consist of damage-associated molecular patterns (DAMPs), such as histones and dsDNA, that are able to activate both the innate and adaptive immune systems. EETosis is evident in several eosinophilic inflammatory diseases, including eosinophilic chronic rhinosinusitis (ECRS), allergic bronchopulmonary aspergillosis and eosinophilic otitis. Thus, EETosis is an important phenomenon for the induction of eosinophilic inflammation.

**Pathogenic roles of CLs in type 2 inflammation**

Eosinophil granules are a source of various cytokines, chemokines, enzymes, extracellular matrix and growth factors (Fig. 1). These factors include interleukin (IL) IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 and IL-13; interferon (IFN)–γ; tumor necrosis factor (TNF); transforming growth factor (TGF)–β; granulocyte-macrophage colony-stimulating factor (GM-CSF); TNFSF13; CC-chemokine ligand (CCL) 3/MIP-1α; CCL5/RANTES; CCL7/MCP-3; and granulocyte-macrophage colony-stimulating factor receptor 2 (GM-CSFR2) ligands, such as CCL2/MCP-1, CCL5/RANTES, and CCL7/MCP-3, which play important roles in the induction of eosinophilic inflammation.
CLC formation is dependent on NADPH oxidase activation

When eosinophils die via cytolysis, the proteins released from granules broadly affect the host’s health and disease susceptibility.

Human eosinophils have four cationic and acid granules proteins: major basic protein (MBP1), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO/EPX). MBP-1 disrupts the lipid bilayer membrane of cells, stimulates histamine release from basophils and mast cells and activates neutrophils and platelets. ECP and EDN are ribonucleases that catalyze the degradation of RNA. EPO/EPX exerts cytotoxic effects as a cationic toxin, being able to kill parasites directly. These cationic proteins are critical for the function of eosinophils as cytotoxic secretory products. In addition, they play an important role in the development of eosinophils. For example, deficiency of both MBP-1 and EPO/EPX results in the near-complete loss of eosinophils in peripheral blood and loss of eosinophil progenitors in the bone marrow. Cystatin F, which is expressed in most immune cells and an inhibitor of cystatin protease, is a critical cell survival factor for eosinophils. Indeed, Cystatin F-deficient mice are short-lived with an abnormal granule architecture in eosinophils. Cystatin F-deficient mice show reduced allergic inflammation in the lung but compromised anti-helminth immunity.

As a non-cationic component, CLCs, a cytoplasmic protein, comprise 7%–10% of eosinophil proteins in humans. CLCs were initially identified as eosinophil lysophospholipase but have since been assigned to the galectin superfamily as galectin-10 protein (Gal10). CLC formation is dependent on NADPH oxidase activation and is closely associated with EETosis. During the process of EETosis, intracellular CLCs and soluble Gal10 are released via plasma membrane rupture, and these released CLCs and Gal10 further form extracellular CLCs. Interestingly, Gal10 itself has potential to promote type 2 immunity by activating both innate and adaptive immune responses. Importantly, antibodies against Gal10 can dissolve CLCs in vivo and ameliorate airway inflammation. Thus, dissolving CLCs can be a novel drug target for eosinophilic inflammation. In asthmatics and patients with eosinophilic chronic rhinosinusitis, CLCs are observed in the inflamed tissue, indicating that CLCs are associated with eosinophilic inflammation and may be a biomarker.

IL-5 is a key cytokine for the activation of eosinophils

Eosinophilia represents an increased number of eosinophils in the blood and/or tissue, defined as >500/mm³ of eosinophils in the peripheral blood. IL-5 is a key pro-inflammatory cytokine for the homeostasis and proliferation of eosinophils (Fig. 2). It induces maturation of eosinophils from CD34⁺ hematopoietic progenitor cells in the bone marrow. IL-5 also induces the activation, proliferation, migration and survival of eosinophils in the peripheral tissues. Eotaxin, which is a ligand for CCR3, is also a vital recruitment regulator of eosinophils. IL-5 and Eotaxin cooperatively promote the proliferation and accumulation of eosinophils in the inflamed tissue. Among various type of immune cells, T helper (Th) type 2 (Th2) cells have the ability to produce IL-5. CD4⁺CD62L⁻CXCR3⁻lowCCR4⁻CCR8⁻ memory-type Th2 cells in particular have the ability to produce large amounts of IL-5 following the stimulation of IL-33, so these Th2⁺ memory Th2 cells were named pathogenic Th2 (Tpath2) cells. Memory-type IL-5-producing Tpath2 cells contribute to the pathogenicity of chronic allergic diseases via the induction of the massive infiltration of eosinophils at local inflammatory sites in both humans and mice. Other sources of IL-5 include group 2 innate lymphoid cells (ILC2s), natural killer (NK) T cells, mast cells and eosinophils. Humanized...
monoclonal antibody against either IL-5 (Mepolizumab) and IL-5 receptor α (Benralizumab) help ameliorate type 2 inflammation in asthma and eosinophilic polyangiitis granulomatosis. These agents decrease in vivo eosinophils significantly and consequently reduce asthma exacerbation accompanied by a reduction in the dose of systemic steroids and improvement of asthma symptoms.44–46

Several subpopulations of eosinophils in vivo

Accumulating evidence has indicated that eosinophils not only shape the pathogenicity during the inflammatory state but also help to regulate the homeostatic processes of various organs in the steady state in vivo.47 A flow cytometry analysis of eosinophils isolated from normal non-lymphoid tissue in mice showed different expression patterns of cell surface molecules, including CD11b, CD11c and Ccr3, in each organ.11 For example, lung-resident homeostatic eosinophils express Siglec-Fmid CD62L CD101low in mice and Siglec-8 mid CD62Lhigh IL-3Rlow in humans.47 In contrast, recruited inflammatory eosinophils express SiglecFhighCD62Llow CD101high in mice and Siglec-8 low CD62Llow IL-3Rhigh in humans.47 Of note, microarray analyses revealed that lung-resident homeostatic eosinophils express several genes, such as Anxa1, Serpinb1a, Runx3 and Nedd4, that are related to the maintenance of immune homeostasis in the lung.48 Indeed, Δ/dbGATA mice, which have no eosinophils genetically, exhibit enhanced sensitivity to the stimulation of allergens, such as house dust mites,48 suggesting that lung-resident homeostatic eosinophils have the ability to regulate type 2 inflammation in the lung.

Eosinophils play a profound role in tissue repair and regeneration

The type 2 immune response is crucial for the tissue repair.49 During the type 2 immune-inflammation, eosinophils play crucial roles in tissue regeneration.50 Muscle damage promotes rapid recruitment of eosinophils in the inflammatory foci. Eosinophils play a key role in muscle regeneration during muscle injury as a major source of IL-4. IL-4 produced by eosinophils activates muscle-resident fibrocyte-adipocyte progenitors (FAPs), which induce regeneration of injured muscles. Type 2 inflammatory cytokine stimulation causes the proliferation of FAPs and induces myogenesis while inhibiting differentiation into adipocytes.

The liver has a substantial ability to regenerate tissue after various types of injury. Eosinophil-derived IL-4 is also required for liver regeneration.51 Eosinophils are recruited in the liver after injury and secrete IL-4 in the local tissue. Eosinophil-derived IL-4 induces the proliferation of quiescent hepatocytes and regulates the regeneration of the liver. Thus, eosinophils play a profound role in tissue repair and regeneration in various organs.

Eosinophils are involved in airway fibrosis

Abnormal tissue repair can induce tissue fibrosis. Excessive deposition of extracellular matrix in the parenchyma causes fibrosis in various organs, which often leads to irreversible organ malfunction. Chronic inflammation of the airway causes the development of pathogenic tissue remodeling, including mucous gland hyperplasia and airway fibrosis. Various stimuli, such as exposure of allergens and virus infection, injure the bronchus and induce the release of epithelial cytokines, including IL-25, IL-33, and TSLP. These epithelial cytokines stimulate eosinophils in both direct and indirect manners and the activated eosinophils are involved in the pathogenesis of airway fibrosis.52 Eosinophils and innate lymphoid cell (ILC)2 express high levels of the IL-33 receptor ST2, and the direct administration of IL-33 into the trachea causes the massive infiltration of eosinophils into the lung.40,53 CD44hiCD62Llow CXCR3lowCCR4 high IL-7Rα hi ST2hi memory-type Tpath2 cells are another target of IL-33 in the inflamed lung.32 As mentioned before, IL-33-stimulated ST2hi memory TH2 cells have the ability to produce large amounts of IL-5, which recruits eosinophils from the blood vessels. Another subpopulation of ST2hi

Fig. 3. The mechanism underlying the airway fibrotic response induced by inflammatory eosinophils. ST2hi memory TH2 cells secrete amphiregulin. Through amphiregulin stimulation, eosinophils activate and produce osteopontin, a key profibrotic inflammatory protein.
memory Th2 cells can produce amphiregulin, an epithelial growth factor (EGF) family member. ST2 expression on memory Th2 cells-derived amphiregulin reprograms eosinophils into inflammatory eosinophils that secrete osteopontin, an extracellular matrix protein. Its deposition leads to fibrosis in both mice and humans. Indeed, amphiregulin stimulation induces the increased expression of genes like CD101 that are related to inflammatory eosinophils.

Chronic rhinosinusitis (CRS) is a chronic inflammatory disease of the sinus associated with characteristic cytokine production profiles and airway remodeling patterns. CRS with polyposis can be classified as either eosinophilic CRS (ECRS) or non-eosinophilic CRS (NECRS) based on histological changes. Patients with ECRS often suffer from asthma, and their polypos exhibit eosinophil infiltration, suggesting that aberrant type 2 immune responses are involved in the pathogenesis of ECRS. Interestingly, a subpopulation of CD45RO+ memory-type CD4+ T cells, CRTH2+/CD161+CD45RO+CD4+ T cells from nasal polyps with ECRS show the high production of amphiregulin. Eosinophils from polypos of ECRS patients show an elevated expression of osteopontin compared to those from the peripheral blood of the same patient. Thus, the IL-33-amphiregulin-osteopontin axis directs fibrotic responses in airway inflammation with the enriched infiltration of eosinophils in both mice and humans (Fig. 3).

Closing remarks

We reviewed the development of eosinophils along with the inflammatory characteristics of these unique granulocytes. We also described recent discoveries regarding the diverse functions of eosinophils in vivo. Eosinophils have both homeostatic and pathogenetic aspects, as seen in their involvement in protective immunity versus allergic inflammation and tissue repair versus fibrosis. Furthermore, Gal10 and amphiregulin may be viable new targets of effective treatment for intractable allergic diseases. To develop new therapeutic strategies for intractable allergic diseases, it will become increasingly important for allergologists to understand the precise features of the pathogenesis of type 2 inflammation induced by various types of immune cells, including eosinophils and Tpath cells.

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Conflict of interest

The authors have no conflict of interest to declare.

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