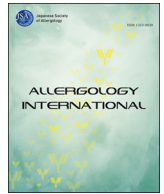




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Invited Review Article

Eosinophils: Cells known for over 140 years with broad and new functions

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ABSTRACT

Eosinophils are multifunctional leukocytes, being involved in the host defense against helminth infection, tissue homeostasis and repair of injured tissue. However, eosinophils also play critical roles in shaping the pathogenesis of allergic diseases, including fibrotic responses in allergic diseases. Eosinophils consist of various granules that are a source of cytokines, chemokines, enzymes, extracellular matrix and growth factors. Recent studies have revealed that eosinophil extracellular trap cell death (EETosis) exacerbates eosinophilic inflammation by releasing the products, including Charcot-Leyden crystals (CLCs). In type 2 inflammatory diseases, memory-type pathogenic helper T (T_{path}) cells are involved in shaping the pathogenesis of eosinophilic inflammation by recruiting and activating eosinophils *in vivo*. We herein review the molecular mechanisms underlying the development of eosinophils and the various functions of granules, including CLCs, during eosinophilic inflammation. We also discuss the double-edged roles of eosinophils in tissue repair and type 2 immune inflammation.

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Introduction

In 1879, German scientist Paul Ehrlich identified eosinophils in the blood of several different mammals, including dogs, rabbits and humans, for the first time.¹ 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.² 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 EETosis (EETosis) and the various function of granules, including CLCs, in eosinophilic inflammation. Finally, we will discuss the double-edged roles of eosinophils in tissue repair and pathogenic remodeling, such as fibrosis, during 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.³ CD34⁺ stem cells proceed with development through granulocyte/macrophage progenitor (GMP) in mice and common myeloid progenitor (CMP) in humans.³ 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 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.² Among these transcription factors, GATA-1 is the key molecule for eosinophil lineage specification because

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eosinophils are deficient in mice with a deletion of the high-affinity GATA-binding site in the GATA-1 promoter region.⁴ Another transcription factor, X-box binding protein 1 (XBP1), and its upstream activator inositol-requiring enzyme 1 α (IRE1 α) modulate the development of eosinophils but not that of other granulocytes, including basophils and neutrophils.⁵ 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.^{6–8}

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-5R α subunit and a β c subunit. Interestingly, IL-5 specifically binds to IL-5R α and activates Janus kinase 2 (Jak2). The β c subunit is also associated with IL-3R α and granulocyte-macrophage colony-stimulating factor receptor α (GM-CSFR α). IL-5, IL-3 and GM-CSF cooperate to promote the proliferation, development and activation of eosinophils.^{9,10}

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.^{3,11} 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 lectin 8 (Siglec-8, mice paralogue; Siglec-F), IL-5 receptor subunit α (IL-5R α) and IFN γ receptor α -chain (IFNGR1). Importantly, eosinophils have the function of antigen presentation against bacteria, virus and helminths.^{12,13} 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.¹⁴ 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.¹⁴

Eosinophil extracellular trap cell death (EETosis) exacerbates 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 (ETosis), which differs from the processes of accidental necrosis or apoptosis.¹⁵ Eosinophil ETosis (EETosis) 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.¹⁵ 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.^{15–17} Thus, EETosis is an important phenomenon for the induction of eosinophilic inflammation.

Pathogenic roles of CLCs 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 1; granulocyte-macrophage colony-stimulating factor (GM-CSF); TNFSF13; CC-chemokine ligand (CCL) 3/MIP-1 α ; CCL5/RANTES; CCL7/MCP-3;

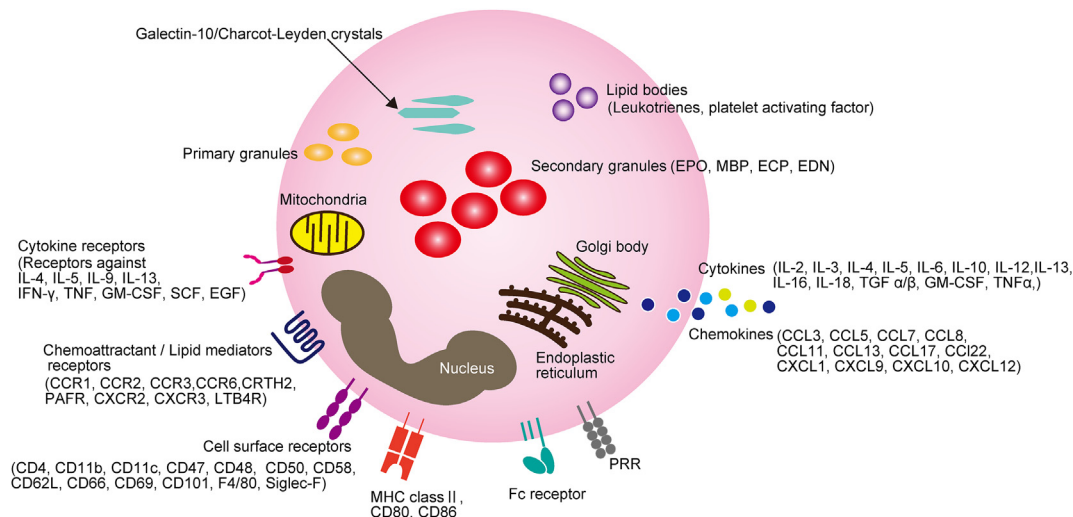


Fig. 1. Features of eosinophils. Eosinophils secrete various types of cytokines, chemokines, lipid bodies and primary/secondary granules. Secondary granules contain eosinophil peroxidase (EPO), major basic protein 1 (MBP), eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN). Eosinophils express a variety of cell surface molecules, such as cytokine receptors, chemokine receptors, Fc receptor and MHC class II.

CCL8/MCP-2; CCL11/Eotaxin-1; CCL13/MCP-4; CCL17/TARC; CCL22/MDC; C-X-C motif ligand (CXCL)1/GRP- α ; CXCL9 MIG; CXCL10/IP-10; CXCL12/SDF-1; elastase; osteopontin; VEGF; PDGF and HB-EGF-LBP.^{11,18,19} 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 1 (MBP1), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO/EPX).^{20–22} MBP-1 disrupts the lipid bilayer membrane of cells, stimulates histamine release from basophils and mast cells and activates neutrophils and platelets.²³ ECP and EDN are ribonucleases that catalyze the degradation of RNA.²⁴ 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.²⁵ 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.²⁶ Cystatin F-deficient mice show reduced allergic inflammation in the lung but compromised anti-helminth immunity.²⁶

As a non-cationic component, CLCs, a cytoplasmic protein, comprise 7%–10% of eosinophil proteins in humans.²⁷ CLCs were initially identified as eosinophil lysophospholipase but have since been assigned to the galectin superfamily as galectin-10 protein (Gal10).²⁷ CLC formation is dependent on NADPH oxidase activation and is closely associated with EETosis.²⁸ 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.²⁸ Interestingly, Gal10 itself has potential to promote type 2 immunity by activating both innate and adaptive immune responses.²⁹ Importantly, antibodies against Gal10 can dissolve CLCs *in vivo* and ameliorate airway inflammation.²⁹ 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 $>500/\text{mm}^3$ of eosinophils in the peripheral blood. IL-5 is a key pro-inflammatory cytokine for the homeostasis and proliferation of eosinophils (Fig. 2).³⁰ 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.^{30,31} 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. CD44^{hi}CD62L^{low}CXCR3^{low}CCR4⁺CCR8⁺IL-7R α ⁺ST2^{hi} memory-type Th2 cells in particular have the ability to produce large amounts of IL-5 following the stimulation of IL-33, so these ST2⁺ memory Th2 cells were named pathogenic Th2 (Tpath2) cells.³² 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.^{33–39} Other sources of IL-5 include group 2 innate lymphoid cells (ILC2s), natural killer (NK) T cells, mast cells and eosinophils.^{40–43} Humanized

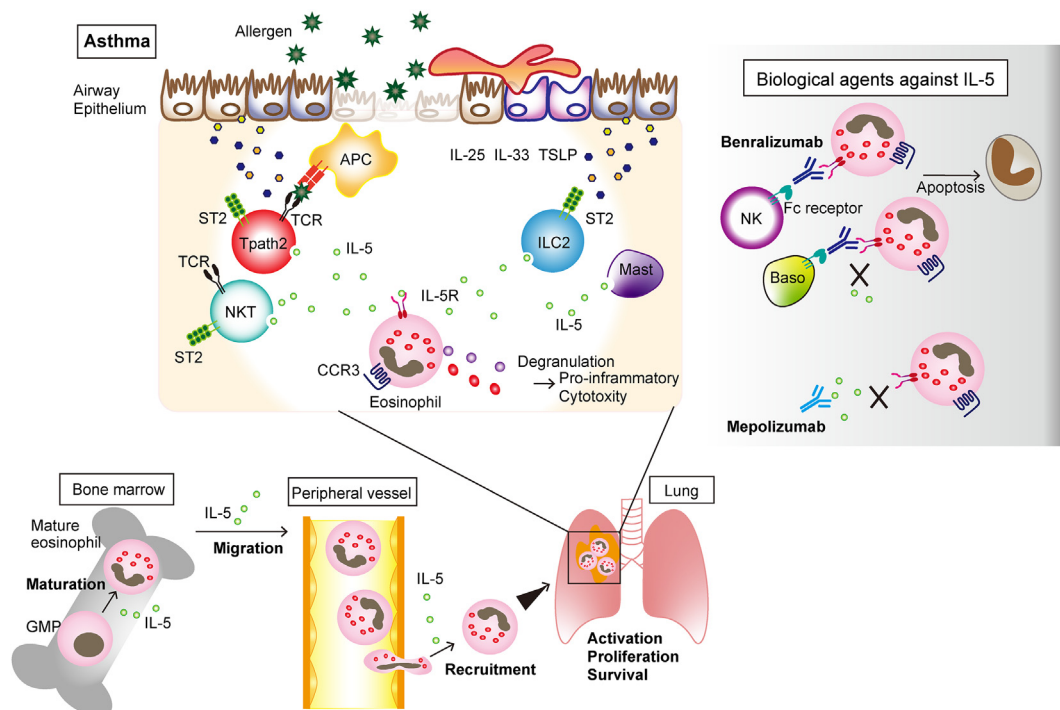


Fig. 2. The broad roles of IL-5 in the development and function of eosinophils. Eosinophils develop in the bone marrow. IL-5 promotes the maturation of eosinophils from granulocyte-macrophage progenitors (GMP) to mature eosinophils. The migration of eosinophils from the bone marrow to the blood is also regulated by IL-5. Eosinophils are recruited to inflamed lung by IL-5 during eosinophilic inflammation. Under conditions of inflammation, IL-5 is mainly produced by Tpath2 cells, ILC2s, Mast cells and NKT cells. Mepolizumab is a humanized monoclonal antibody against IL-5 that inhibits the biological activity against eosinophils. Benralizumab is a humanized monoclonal antibody against IL-5 receptor α that induces the apoptosis of eosinophils.

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 symptom.^{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-F^{mid}CD62L⁺CD101^{low} in mice and Siglec-8⁺CD62L^{hi}IL-3R^{low} in humans.⁴⁷ In contrast, recruited inflammatory eosinophils express Siglec-F^{high}CD62L^{low}CD101^{high} in mice and Siglec-8⁺CD62L^{low}IL-3R^{high} in humans.⁴⁷ Of note, microarray analyses revealed that lung-resident homeostatic eosinophils express several genes, such as *Anxa1*, *Serpinb1a*, *Runx3* and *Nedda4*, that are related to the maintenance of immune homeostasis in the lung.⁴⁸ Indeed, Δ b1GATA 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} CD44^{hi}CD62L^{low}CXCR3^{low}CCR4⁺CCR8⁺IL-7R α ⁺ST2^{hi} memory-type Tpath2 cells are another target of IL-33 in the inflamed lung.³² As mentioned before, IL-33-stimulated ST2^{hi} memory Th2 cells have the ability to produce large amounts of IL-5, which recruits eosinophils from the blood vessels. Another subpopulation of ST2^{hi}

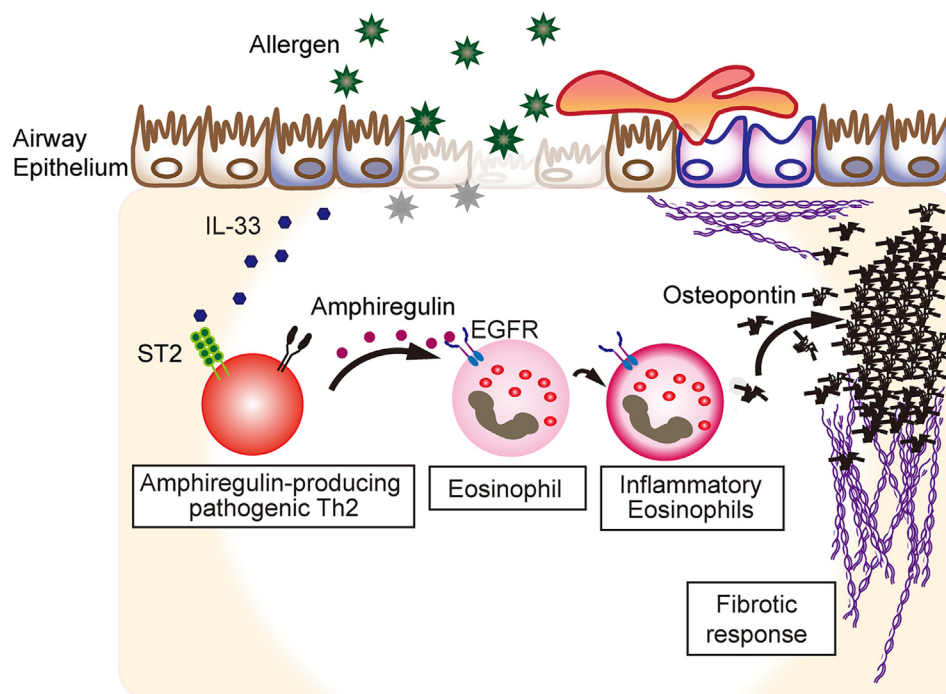


Fig. 3. The mechanism underlying the airway fibrotic response induced by inflammatory eosinophils. ST2^{high} memory Th2 cells secrete amphiregulin. Through amphiregulin stimulation, eosinophils activate and produce osteopontin, a key profibrotic inflammatory protein.

memory Th2 cells can produce amphiregulin, an epithelial growth factor (EGF) family member.⁵⁴ ST2^{high} memory Th2 cell-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.^{48,51}

Chronic rhinosinusitis (CRS) is a chronic inflammatory disease of the sinus associated with characteristic cytokine production profiles and airway remodeling patterns.⁵⁵ CRS with polyps can be classified as either eosinophilic CRS (ECRS) or non-eosinophilic CRS (NECRS) based on histological changes.⁵⁶ Patients with ECRS often suffer from asthma, and their polyps exhibit eosinophilic infiltration, suggesting that aberrant type 2 immune responses are involved in the pathogenesis of ECRS.⁵⁶ Interestingly, a subpopulation of CD45RO⁺ memory-type CD4⁺ T cells, CRTH2^{high}CD161^{high} CD4⁺ T cells from nasal polyps with ECRS show the high production of amphiregulin. Eosinophils from polyps 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.

References

- Bystrom J, Amin K, Bishop-Bailey D. Analysing the eosinophil cationic protein – a clue to the function of the eosinophil granulocyte. *Respir Res* 2011;**12**:10.
- Rothenberg ME, Hogan SP. The eosinophil. *Annu Rev Immunol* 2006;**24**:147–74.
- Rosenberg HF, Dyer KD, Foster PS. Eosinophils: changing perspectives in health and disease. *Nat Rev Immunol* 2013;**13**:9–22.
- Yu C, Cantor AB, Yang H, Browne C, Wells RA, Fujiwara Y, et al. Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage *in vivo*. *J Exp Med* 2002;**195**:1387–95.
- Bettigole SE, Lis R, Adoro S, Lee AH, Spencer LA, Weller PF, et al. The transcription factor XBP1 is selectively required for eosinophil differentiation. *Nat Immunol* 2015;**16**:829–37.
- Yamaguchi Y, Nishio H, Kishi K, Ackerman SJ, Suda T. C/EBPβ and GATA-1 synergistically regulate activity of the eosinophil granule major basic protein promoter: implication for C/EBPβ activity in eosinophil gene expression. *Blood* 1999;**94**:1429–39.
- Shahrin NH, Diakow S, Dent LA, Brown AL, D'Andrea RJ. Conditional knockout mice demonstrate function of Klf5 as a myeloid transcription factor. *Blood* 2016;**128**:55–9.
- Buitenhuis M, van Deutekom HW, Verhagen LP, Castor A, Jacobsen SE, Lammers JW, et al. Differential regulation of granulopoiesis by the basic helix-loop-helix transcriptional inhibitors Id1 and Id2. *Blood* 2005;**105**:4272–81.
- Lopez AF, Begley CG, Williamson DJ, Warren DJ, Vadas MA, Sanderson CJ. Murine eosinophil differentiation factor. An eosinophil-specific colony-stimulating factor with activity for human cells. *J Exp Med* 1986;**163**:1085–99.
- Rothenberg ME, Pomerantz JL, Owen Jr WF, Avraham S, Soberman RJ, Austen KF, et al. Characterization of a human eosinophil proteoglycan, and augmentation of its biosynthesis and size by interleukin 3, interleukin 5, and granulocyte/macrophage colony stimulating factor. *J Biol Chem* 1988;**263**:13901–8.
- Weller PF, Spencer LA. Functions of tissue-resident eosinophils. *Nat Rev Immunol* 2017;**17**:746–60.
- Shi HZ. Eosinophils function as antigen-presenting cells. *J Leukoc Biol* 2004;**76**:520–7.
- Akuthota P, Wang HB, Spencer LA, Weller PF. Immunoregulatory roles of eosinophils: a new look at a familiar cell. *Clin Exp Allergy* 2008;**38**:1254–63.
- Kiwamoto T, Katoh T, Evans CM, Janssen WJ, Brummet ME, Hudson SA, et al. Endogenous airway mucins carry glycans that bind Siglec-F and induce eosinophil apoptosis. *J Allergy Clin Immunol* 2015;**135**:1329–40.e9.
- Ueki S, Melo RC, Ghiran I, Spencer LA, Dvorak AM, Weller PF. Eosinophil extracellular DNA trap cell death mediates lytic release of free secretion-competent eosinophil granules in humans. *Blood* 2013;**121**:2074–83.
- Ueki S, Konno Y, Takeda M, Moritoki Y, Hirokawa M, Matsuwaki Y, et al. Eosinophil extracellular trap cell death-derived DNA traps: their presence in secretions and functional attributes. *J Allergy Clin Immunol* 2016;**137**:258–67.
- Muniz VS, Silva JC, Braga YAV, Melo RCN, Ueki S, Takeda M, et al. Eosinophils release extracellular DNA traps in response to *Aspergillus fumigatus*. *J Allergy Clin Immunol* 2018;**141**:571–85.e7.
- Spencer LA, Szela CT, Perez SA, Kirchhoffer CL, Neves JS, Radke AL, et al. Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially. *J Leukoc Biol* 2009;**85**:117–23.
- Rose Jr CE, Lannigan JA, Kim P, Lee JJ, Fu SM, Sung SS. Murine lung eosinophil activation and chemokine production in allergic airway inflammation. *Cell Mol Immunol* 2010;**7**:361–74.
- Ackerman SJ, Kephart GM, Habermann TM, Greipp PR, Gleich GJ. Localization of eosinophil granule major basic protein in human basophils. *J Exp Med* 1983;**158**:946–61.
- Egesten A, Alumets J, von Mecklenburg C, Palmegren M, Olsson I. Localization of eosinophil cationic protein, major basic protein, and eosinophil peroxidase in human eosinophils by immunoelectron microscopic technique. *J Histochem Cytochem* 1986;**34**:1399–403.
- Peters MS, Rodriguez M, Gleich GJ. Localization of human eosinophil granule major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin by immunoelectron microscopy. *Lab Invest* 1986;**54**:656–62.
- Gundel RH, Letts LG, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J Clin Invest* 1991;**87**:1470–3.
- Ackerman SJ, Loegering DA, Venge P, Olsson I, Harley JB, Fauci AS, et al. Distinctive cationic proteins of the human eosinophil granule: major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin. *J Immunol* 1983;**131**:2977–82.
- Doyle AD, Jacobsen EA, Ochkur SI, McGarry MP, Shim KG, Nguyen DT, et al. Expression of the secondary granule proteins major basic protein 1 (MBP-1) and eosinophil peroxidase (EPX) is required for eosinophilopoiesis in mice. *Blood* 2013;**122**:781–90.
- Matthews SP, McMillan SJ, Colbert JD, Lawrence RA, Watts C, Cystatin F. Ensures eosinophil survival by regulating granule biogenesis. *Immunity* 2016;**44**:795–806.
- Acharya KR, Ackerman SJ. Eosinophil granule proteins: form and function. *J Biol Chem* 2014;**289**:17406–15.
- Ueki S, Tokunaga T, Melo RCN, Saito H, Honda K, Fukuchi M, et al. Charcot-Leyden crystal formation is closely associated with eosinophil extracellular trap cell death. *Blood* 2018;**132**:2183–7.
- Persson EK, Verstraete K, Heyndrickx I, Gevaert E, Aegerter H, Percier JM, et al. Protein crystallization promotes type 2 immunity and is reversible by antibody treatment. *Science* 2019;**364**:eaaw4295.
- Takatsu K, Nakajima H. IL-5 and eosinophilia. *Curr Opin Immunol* 2008;**20**:288–94.
- Shakoori B, Fitzgerald SM, Lee SA, Chi DS, Krishnaswamy G. The role of human mast cell-derived cytokines in eosinophil biology. *J Interferon Cytokine Res* 2004;**24**:271–81.

32. Nakayama T, Hirahara K, Onodera A, Endo Y, Hosokawa H, Shinoda K, et al. Th2 cells in health and disease. *Annu Rev Immunol* 2017;**35**:53–84.
33. Endo Y, Hirahara K, Iinuma T, Shinoda K, Tumes DJ, Asou HK, et al. The interleukin-33-p38 kinase axis confers memory T helper 2 cell pathogenicity in the airway. *Immunity* 2015;**42**:294–308.
34. Iinuma T, Okamoto Y, Yamamoto H, Inamine-Sasaki A, Ohki Y, Sakurai T, et al. Interleukin-25 and mucosal T cells in noneosinophilic and eosinophilic chronic rhinosinusitis. *Ann Allergy Asthma Immunol* 2015;**114**:289–98.
35. Islam SA, Chang DS, Colvin RA, Byrne MH, McCully ML, Moser B, et al. Mouse CCL8, a CCR8 agonist, promotes atopic dermatitis by recruiting IL-5+ T(H)2 cells. *Nat Immunol* 2011;**12**:167–77.
36. Mitsun-Salazar A, Yin Y, Wansley DL, Young M, Bolan H, Arceo S, et al. Hematopoietic prostaglandin D synthase defines a proeosinophilic pathogenic effector human TH2 cell subpopulation with enhanced function. *J Allergy Clin Immunol* 2016;**137**:907–18.e9.
37. Upadhyaya B, Yin Y, Hill BJ, Douek DC, Prussin C. Hierarchical IL-5 expression defines a subpopulation of highly differentiated human Th2 cells. *J Immunol* 2011;**187**:3111–20.
38. Wambre E, DeLong JH, James EA, LaFond RE, Robinson D, Kwok WW. Differentiation stage determines pathologic and protective allergen-specific CD4+ T-cell outcomes during specific immunotherapy. *J Allergy Clin Immunol* 2012;**129**:544–51. 51 e1–7.
39. Wambre E, Bajzik V, DeLong JH, O'Brien K, Nguyen QA, Speake C, et al. A phenotypically and functionally distinct human TH2 cell subpopulation is associated with allergic disorders. *Sci Transl Med* 2017;**9**:eaam9171.
40. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature* 2010;**463**:540–4.
41. Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. *Immunity* 2019;**50**:975–91.
42. Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P, et al. Eosinophils: biological properties and role in health and disease. *Clin Exp Allergy* 2008;**38**:709–50.
43. Sakuishi K, Oki S, Araki M, Porcelli SA, Miyake S, Yamamura T. Invariant NKT cells biased for IL-5 production act as crucial regulators of inflammation. *J Immunol* 2007;**179**:3452–62.
44. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;**360**:973–84.
45. Bel EH, Wenzel SE, Thompson PJ, Prazma CM, Keene ON, Yancey SW, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 2014;**371**:1189–97.
46. Bleecker ER, FitzGerald JM, Chanez P, Papi A, Weinstein SF, Barker P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting beta2-agonists (SIRIOCCO): a randomised, multicentre, placebo-controlled phase 3 trial. *Lancet* 2016;**388**:2115–27.
47. Marichal T, Mesnil C, Bureau F. Homeostatic eosinophils: characteristics and functions. *Front Med* 2017;**4**:101.
48. Mesnil C, Raulier S, Paulissen G, Xiao X, Birrell MA, Pirotton D, et al. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. *J Clin Invest* 2016;**126**:3279–95.
49. Gieseck 3rd RL, Wilson MS, Wynn TA. Type 2 immunity in tissue repair and fibrosis. *Nat Rev Immunol* 2018;**18**:62–76.
50. Heredia JE, Mukundan L, Chen FM, Mueller AA, Deo RC, Locksley RM, et al. Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. *Cell* 2013;**153**:376–88.
51. Goh YP, Henderson NC, Heredia JE, Red Eagle A, Odegaard JI, Lehwald N, et al. Eosinophils secrete IL-4 to facilitate liver regeneration. *Proc Natl Acad Sci U S A* 2013;**110**:9914–9.
52. Hirahara K, Aoki A, Morimoto Y, Kiuchi M, Okano M, Nakayama T. The immunopathology of lung fibrosis: amphiregulin-producing pathogenic memory T helper-2 cells control the airway fibrotic responses by inducing eosinophils to secrete osteopontin. *Semin Immunopathol* 2019;**41**:339–48.
53. Mato N, Hirahara K, Ichikawa T, Kumagai J, Nakayama M, Yamasawa H, et al. Memory-type ST2(+)CD4(+) T cells participate in the steroid-resistant pathology of eosinophilic pneumonia. *Sci Rep* 2017;**7**:6805.
54. Morimoto Y, Hirahara K, Kiuchi M, Wada T, Ichikawa T, Kanno T, et al. Amphiregulin-producing pathogenic memory T helper 2 cells instruct eosinophils to secrete osteopontin and facilitate airway fibrosis. *Immunity* 2018;**49**:134–50.e6.
55. Bal SM, Bernink JH, Nagasawa M, Groot J, Shikhagaie MM, Golebski K, et al. IL-1beta, IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs. *Nat Immunol* 2016;**17**:636–45.
56. Hamilos DL. Drivers of chronic rhinosinusitis: inflammation versus infection. *J Allergy Clin Immunol* 2015;**136**:1454–9.