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Invited Review Article

The multiple functions and subpopulations of eosinophils in tissues under steady-state and pathological conditions

Akira Kanda ^{a, b, c, *}, Yasutaka Yun ^{a, 1}, Dan Van Bui ^{a, 1}, Linh Manh Nguyen ^a, Yoshiki Kobayashi ^{a, b}, Kensuke Suzuki ^a, Akitoshi Mitani ^a, Shunsuke Sawada ^a, Satoko Hamada ^a, Mikiya Asako ^{a, b}, Hiroshi Iwai ^a^a Department of Otolaryngology, Head and Neck Surgery, Kansai Medical University, Osaka, Japan^b Allergy Center, Kansai Medical University, Osaka, Japan^c Department of Pathology and Laboratory Medicine, Kansai Medical University, Osaka, Japan

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ABSTRACT

Eosinophils not only play a critical role in the pathogenesis of eosinophil-associated diseases, but they also have multiple important biological functions, including the maintenance of homeostasis, host defense against infections, immune regulation through canonical Th1/Th2 balance modulation, and anti-inflammatory and anti-tumorigenic activities. Recent studies have elucidated some emerging roles of eosinophils in steady-state conditions; for example, eosinophils contribute to adipose tissue metabolism and metabolic health through alternatively activated macrophages and the maintenance of plasma cells in intestinal tissue and bone marrow. Moreover, eosinophils exert tissue damage through eosinophil-derived cytotoxic mediators that are involved in eosinophilic airway inflammation, leading to diseases including asthma and chronic rhinosinusitis with nasal polyps characterized by fibrin deposition through excessive response by eosinophils-induced. Thus, eosinophils possessing these various effects reflect the heterogeneous features of these cells, which suggests the existence of distinct different subpopulations of eosinophils between steady-state and pathological conditions. Indeed, a recent study demonstrated that instead of dividing eosinophils by classical morphological changes into normodense and hypodense eosinophils, murine eosinophils from lung tissue can be phenotypically divided into two distinct subtypes: resident eosinophils and inducible eosinophils gated by Siglec-F^{int} CD62L⁺ CD101^{low} and Siglec-F^{high} CD62L[−] CD101^{high}, respectively. However, it is difficult to explain every function of eosinophils by rEos and iEos, and the relationship between the functions and subpopulations of eosinophils remains controversial. Here, we overview the multiple roles of eosinophils in the tissue and their biological behavior in steady-state and pathological conditions. We also discuss eosinophil subpopulations.

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Introduction

Since Ehrlich first mentioned cells stained by eosin in 1879,¹ several studies have reported on the multiple biological aspects of eosinophils including their beneficial, detrimental, regulatory, and homeostatic effects.^{2–5} However, the intrinsic roles of eosinophils are much more complex. During eosinophil development in the bone marrow (BM), eosinophil progenitor (EoP) cells expressed

with IL-5R α arise from a common myeloid progenitor and develop into mature eosinophils by cytokine signaling, primarily by IL-5. This process involves a complex hierarchically expressed network of transcription factors such as PU.1, FOG1, C/EBP family members, GATA1, GATA2, XBP1, and ID2 (Fig. 1A).⁶ Circulating eosinophils from the BM transmigrate into the tissue with adhesion receptors from the integrin family (e.g., $\alpha 4\beta 1$, $\alpha 4\beta 7$, $\alpha 6\beta 1$, $\alpha D\beta 2$, $\alpha L\beta 2$, $\alpha M\beta 2$, and $\alpha X\beta 2$).⁷ This infiltration process from the bloodstream into the tissue is associated with the maturation of eosinophils and arises from a large variety of receptors on eosinophils such as chemoattractant receptors, cytokine and growth factor receptors, adhesion receptors, pattern-recognition receptors, Fc receptors, and receptors for lipid mediators (Table 1).^{2–5} Eosinophils can then exert multiple effects using a variety of eosinophil-derived mediators through the signaling of these receptors (Table 2).^{2–5} Thus,

* Corresponding author. Department of Otolaryngology, Head and Neck Surgery, Kansai Medical University, 2-5-1, Shinmachi, Hirakata City, Osaka, 573-1010, Japan.

E-mail address: akanda@hirakata.kmu.ac.jp (A. Kanda).

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¹ These authors contributed equally to this study.

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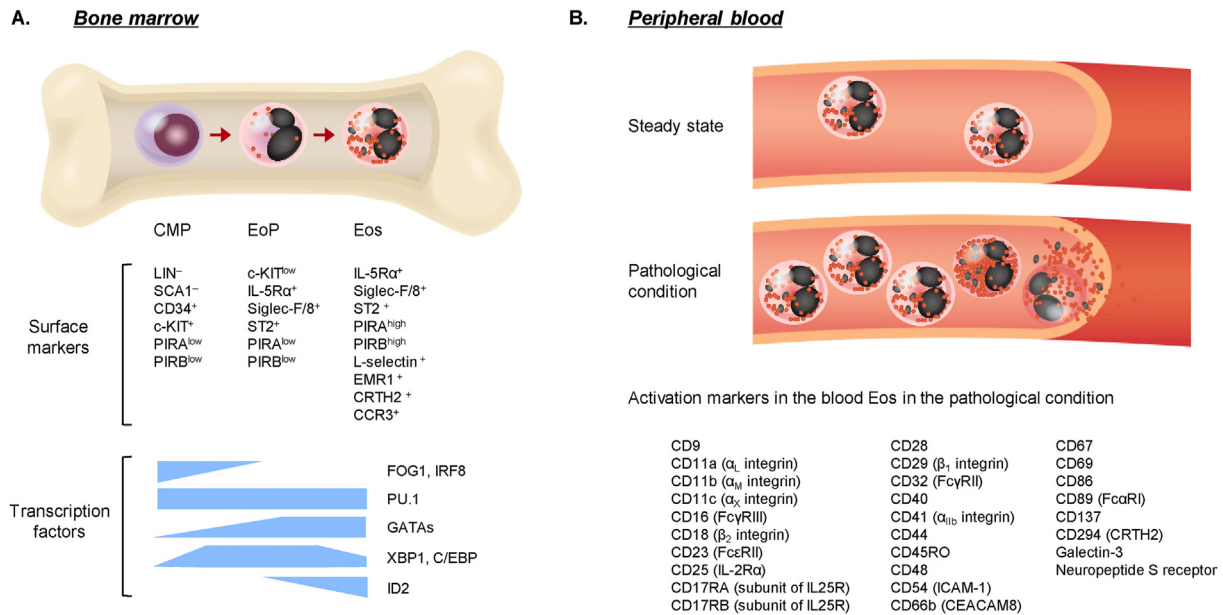


Fig. 1. Eosinophil maturation in the bone marrow and activation markers in the peripheral blood. **A.** The differentiation of eosinophils from common myeloid progenitor (CMP) cells and eosinophil progenitors (EoP) to mature eosinophils (Eos). The corresponding surface expression and transcriptional factors in CMP, EoP, and Eos are shown below; for detailed information, see references^{6,68}. **B.** The peripheral blood in the steady-state and pathological conditions. For more details about activation markers, see references^{62,79–81}. C/EBP, CCAAT/enhancer-binding protein; CEACAM, carcinoembryonic antigen; CRTH2, chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells; EMR1, epidermal growth factor-like module-containing, mucin-like hormone receptor 1; GATA-1 and -2, GATA Binding Protein 1 and 2; Lin, lineage; PIRA, paired immunoglobulin-like receptor A; PIRB, paired immunoglobulin-like receptor B.

Table 1
Receptors in eosinophils.

Adhesion receptors and others	: CD34, CD44, CD54 (ICAM-1), CD62L (L-selectin), CD69, CR3 (Mac1; CD11b–CD18), CR4 (CD11c–CD18), LFA (CD11a–CD18), PSGL1, VLA4 (CD49d–CD29), 2B4 (CD244)
Chemokine receptors	: CCR1, CCR3, CCR4, CCR5, CCR6, CCR8, CCR9, CXCR2, CXCR3, CXCR4, FPR1
Complement receptors	: C5aR, C3aR
Costimulatory receptors	: CD28, CD86
Cytokine receptors	: IL-2R, IL-3R, IL-4R, IL-5R, IL-9R, IL-10R, IL-13R, IL-17R, IL-23R, IL-27R, IL-31R, IL-33R, TSLPR
Fc receptors	: FcαR, FcγRII, FcεRI, FcεRII
Growth factor receptors	: GM-CSFR, c-kit (CD117), IFN-γR, TGF-βR
Inhibitory receptor	: Siglec-8
Lipid mediator receptors	: DP1 prostaglandin receptor, DP2 prostaglandin receptor (CRTH2), EP2 prostaglandin receptor, LTB4, PAFR
MHCII	
Nuclear receptors	: PPAR γ
Paired immunoglobulin-like receptor B (PIRB)	
Pattern-recognition receptors (PRRs)	: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR9, TLR10, NOD1, NOD2, RIGs, RAGE
Proteinase-activated receptors (PARs)	: PAR1, PAR2

FcR, Fc receptors; FPR1, formyl peptide receptor 1; GM-CSFR, granulocyte–macrophage colony-stimulating factor receptor; ICAM-1, intercellular adhesion molecule-1; IFN-γR, interferon γ receptor; LFA-1, lymphocyte function-associated antigen-1; PAFR, platelet-activating factor receptor; PAR, proteinase-activated receptor; PIRB, paired immunoglobulin-like receptor B; PPARγ, peroxisome proliferator-activated receptor-γ; PRR, pattern-recognition receptor; PSGL1, P-selectin glycoprotein ligand 1; RAGE, receptor for advanced glycation end-products; RIG-1, retinoic acid-inducible gene 1; TGF-βR, transforming growth factor β receptor; TSLPR, thymic stromal lymphopoietin receptor; TLR, Toll-like receptor; VLA, very late antigen.

eosinophils are characterized by their numerous receptors and mediators, which suggests that signaling by environmental insults determines these different roles of eosinophils.

Eosinophils have multiple functions; they play an important role in host defense against parasitic, viral, fungal, and bacterial infections through eosinophil-derived cytotoxic mediators such as granule proteins (major basic protein [MBP], eosinophil peroxidase [EPO], eosinophil cationic protein [ECP], and eosinophil-derived neurotoxin [EDN])^{2–5} and/or creating mitochondrial DNA-containing traps in the extracellular space to engulf microorganisms in an instantaneous catapult-like fashion⁸; they also maintain homeostasis in the steady-state.⁹ Moreover, eosinophils contribute to the pathogenesis of allergic airway inflammation (e.g., allergic rhinitis [AR], eosinophilic otitis media, eosinophilic chronic rhinosinusitis [ECRS], and asthma), atopic dermatitis (AD), hyper eosinophilic syndromes (HES), eosinophilic gastrointestinal diseases (EGIDs), and demyelination diseases (e.g., multiple sclerosis and neuromyelitis optica); these reflect some detrimental aspects of eosinophils.^{2–5} Although the mechanisms by which eosinophils display multiple different functions are unclear, this variety appears to be determined by a heterogeneous rather than homogenous cell population with different functions, which suggests the existence of distinct eosinophil subpopulations in healthy and pathological tissue. Indeed, it has historically been known that there are two different specific gravity cell types of eosinophils: those with normal density and those with lower density, the latter of which represents the activated phenotype.^{10,11} Recent studies suggest a new concept about two different subtypes of eosinophils that can be classified by surface expression markers using flow cytometry: these subtypes are resident (rEos) and inducible eosinophils (iEos),

Table 2
Eosinophil-derived mediators.

Cytokines	: IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-16, IL-18, IL-25, GM-CSF, IFN γ , TNF α / β
Chemokines	: CCL2 (MCP), CCL3 (MIP-1 α), CCL4 (MIP-1 β), CCL5 (RANTES), CCL6 (C10), CCL7 (MCP-3), CCL8, CCL11 (eotaxin), CCL13 (MIP-4), CCL17 (TARC), CCL18 (PARC), CCL22 (MDP), CCL24 (eotaxin-2), CXCL1 (GRO α), CXCL5 (ENA), CXCL8 (IL-8), CXCL9 (MIG), CXCL10 (IP-10), CXCL12 (SDF-1 α)
EETs	: extracellular mitochondrial DNA traps
Enzymes	: Acid phosphatase, Arylsulphatase B, Catalase, Collagenase, Nonspecific esterases, Histaminase, IDO, KYN, MMP-9, Phospholipase D
Growth factors and others	: APRIL, EGF, FGF2, NGF, HB-EGF-LBP, PDGF, SCF, TGF α / β 1, VEGF, OPN
Granule proteins	: MBP, ECP, EDN, EPO
Lipid mediators	: LTC4, LTD4, LTE4, TXB2, PGE1, PGE2, 15-HETE, PAF, Protectin D1
Neuro mediators	: NGF, substance P, VIP
Oxidative metabolite	: ROS

APRIL, a proliferation-inducing ligand; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EETs, eosinophil extracellular traps; EGF, epidermal growth factor; EGE, eosinophilic gastroenteritis; ENA, epithelial-derived neutrophil-activating; EPO, eosinophil peroxidase; FGF, fibroblast growth factor; GRO α , growth-related oncogene α ; HB-EGF-LBP, heparin-binding epidermal growth factor-like binding protein; ICAM-1, intercellular adhesion molecule-1; IDO, indoleamine, 2,3-dioxygenase; IP-10, IFN- γ -inducible protein 10; KYN, kynurenine; MBP, major basic protein; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine; MIG, monokine induced by IFN- γ ; MIP, macrophage inflammatory protein; MMP, Matrix metalloproteinase; NGF, nerve growth factor; OPN, osteopontin; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T cell expressed and secreted; PARC, pulmonary and activation regulated chemokine; ROS, radical oxygen; SCF, stem cell factor; TGF, transforming growth factor; TXB, thromboxane B; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal peptide; 15-HETE, 15-Hydroxyeicosatetraenoic acid.

which are located in steady-state and inflamed tissue, respectively.¹² Thus, it is likely that the existence of phenotypically distinct subpopulations of eosinophils is accompanied by multiple biological functions in which eosinophils are considered terminally differentiated cytotoxic effector cells.

Multiple roles of eosinophils in the tissue

A large number of eosinophils reside in the tissue outside the vasculature whereas blood eosinophils comprise a minor (<5%) component of circulating leukocytes.¹³ Eosinophils reside in several organs including the thymus, adipose tissue, intestines, mammary glands, uterus, and lungs under homeostatic conditions.^{9,13} The intestines contain the largest number of tissue rEos in the body per total area (5–25% of total leukocytes); the adipose tissue and lungs contain <4% of the stromal/vascular fraction and <1% of total leukocytes, respectively.^{9,12,14,15} The number of eosinophils fluctuates over time in the uterus, mammary glands, and thymus, and it varies in the uterus and mammary glands depending on sex hormone cycles.^{9,16} Although the half-life of eosinophils in the peripheral blood is only 3–18 h, tissue eosinophils in the intestines, uterus, and lungs exist longer (~6–7 days, ~6 days, and 36 h–6 days, respectively).^{9,17} Eosinophils incubated with cytokines *in vitro* can survive for 14 days or longer.¹⁸ Thus, tissue tropism and the microenvironment can prolong eosinophil survival and seem to determine behavior of eosinophils in the maintenance of homeostasis. As shown in Figure 2, homeostatic eosinophils (hEos) found in the tissue play important roles in metabolic homeostasis, wound healing, epithelial remodeling in the respiratory tract, the maintenance of homeostasis in the intestinal environment, the

maintenance of plasma cells in the BM, mammary gland development, and the modulation of the female genital system; they also play an immunomodulatory role in the thymus.^{9,19,20}

Adipose tissue

An emerging role of eosinophils is their contribution to adipose tissue metabolism and metabolic health through alternatively activated (M2) macrophages (AAMs) that play a crucial role in glucose homeostasis and the development of beige fat, which improves glucose tolerance, insulin reactivity, and protects against obesity.^{15,21–23} Notably, Wu *et al.* showed that rEos in adipose tissue mediate macrophage differentiation into the AAM phenotype by eosinophil-derived IL-4 in white adipose tissue, and mice fed a high-fat diet exhibited impaired glucose tolerance and insulin resistance in the absence of eosinophils; conversely, helminth-induced adipose eosinophilia enhanced glucose tolerance.¹⁵ When assessing the mechanisms of eosinophil accumulation in homeostatic adipose tissue, Molofsky *et al.* revealed that IL-5 and IL-13 from group 2 innate lymphoid cells (ILC2s), which regulate type 2 immune responses by producing large amounts of type 2 cytokines prior to the acquired immune response, increased the number of eosinophils and AAMs in visceral adipose tissue; this effect was enhanced during intestinal helminth infection.²² Moreover, Bolus *et al.* demonstrated that CCR2 deficiency resulted in increased eosinophils, AAM activation, and type 2 cytokine expression in adipose tissue, suggesting that a population of CCR2-expressing cells regulates eosinophils in adipose tissue.²⁴ Collectively, those results indicate that eosinophil-regulated AAMs in adipose tissue may play an important role in metabolic homeostasis.

Although metabolic syndrome and obesity are at an increased risk of developing asthma^{25,26} and bronchial submucosal eosinophils are elevated in obese patients with severe asthma,²⁷ definitive clinical studies demonstrating that increased eosinophils have a protective effect against obesity-related disease in human patients remain controversial. Indeed, conflicting reports have been shown; one study noted a positive correlation between peripheral eosinophil count and the duration of diabetic nephropathy in males with type 2 diabetes²⁸ whereas another reported a negative correlation between the percentage of eosinophils in the peripheral blood and the prevalence of type 2 diabetes in Chinese adults.²⁹ Thus, this topic requires further investigation.

Respiratory tract

There are very few eosinophils in the lungs of mice at birth; they are recruited to the lungs by IL-5 from ILC2 via epithelium-derived IL-33 in response to the “first breath” in the lungs of newborn mice.^{30,31} Sensitivity to house dust mites (HDM) is increased in the lungs of eosinophil-deficient mice¹²; furthermore, activated eosinophils from both aspergillus antigen and cytokine-driven asthma models displayed a protection effect against lethal respiratory virus infection.³² Together, these reports suggest that eosinophils in the lungs contribute to the maintenance of lung immune homeostasis and host defense, respectively. Additionally, eosinophils play important roles in coagulation (tissue factor, thrombin, and fibrinogen) and fibrinolysis, which is a highly regulated enzymatic process that prevents unnecessary accumulation of intravascular fibrin and enables the removal of thrombi, (the plasminogen activation cascade, such as fibrinogen and the urokinase-type plasminogen activator/urokinase-type plasminogen activator receptor system), indicating their contribution to wound healing and epithelial remodeling.¹⁹ Meanwhile, an excessive response from the interaction between eosinophils and dysregulated coagulation is involved in type 2 airway

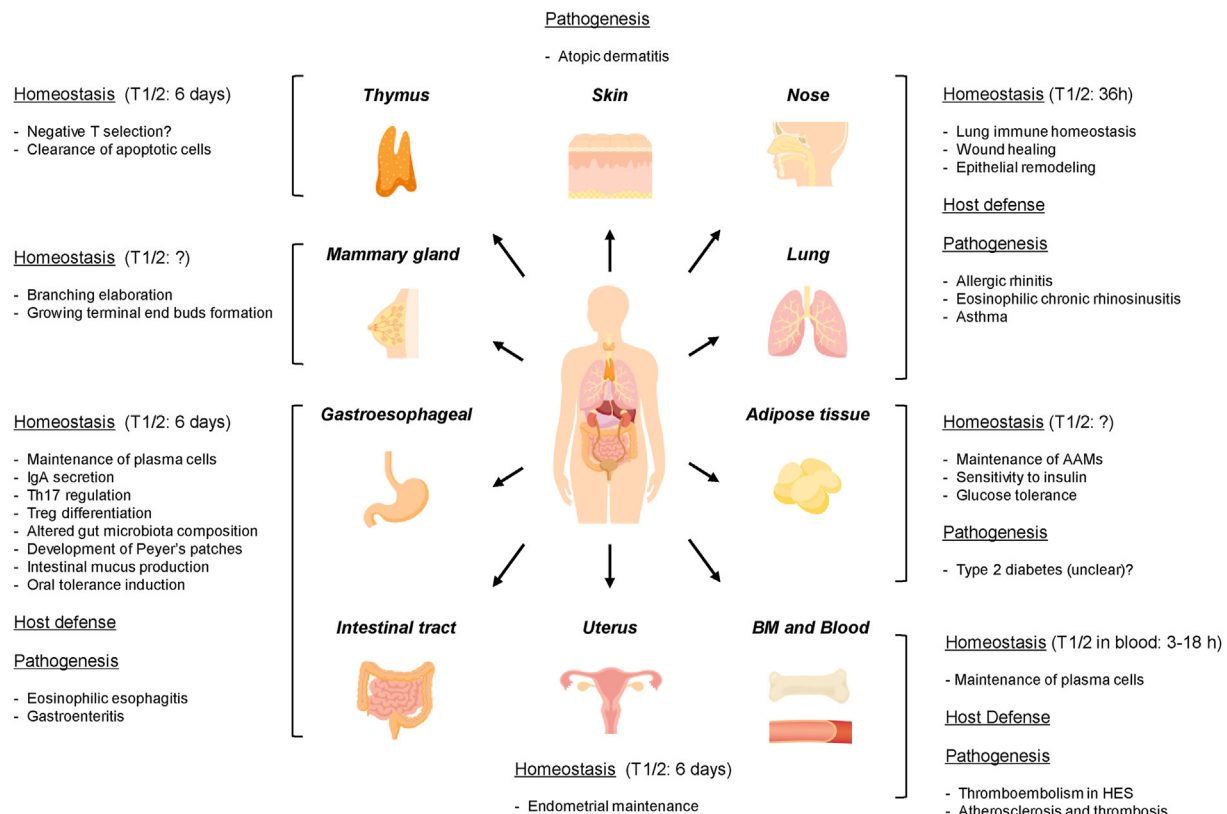


Fig. 2. Schematic overview of the multiple function of eosinophils in the individual organ tissue. AAMs, alternatively activated macrophages; HES, hypereosinophilic syndrome; T1/2, half-life period.

inflammatory diseases including asthma and chronic rhinosinusitis with nasal polyps characterized by fibrin deposition.^{19,33–35}

Gastrointestinal and esophagus

Many eosinophils are commonly found in the gastrointestinal tract and cecum whereas few are located in the esophagus.^{3,4} They mainly reside in the lamina propria but are not found in intra-epithelial locations inside the tissue.^{3,4} These eosinophils play a critical role in the maintenance of intestinal homeostasis and biological defense²⁰; conversely, eosinophils also contribute to the development of EGIDs including eosinophilic esophagitis and gastroenteritis, which are commonly induced by an allergic response to food or environmental allergens through eosinophil-derived mediators (e.g., eotaxin-3, IL-5, IL-13, TGF- β , and perostin).^{36,37} Chu *et al.* reported that eosinophil-deficient mice, specifically Δ dblGATA-1 and PHIL mice, or an eosinophil-specific depletion model showed defects in the intestinal mucous shield, alterations in the microbiota composition in the gut lumen, decreased IgA⁺ B and plasma cell numbers and secretion of IgA, and decreased numbers of regulatory T cells and dendritic cells in gut-associated tissues, suggesting that eosinophils contribute to gut immune homeostasis in the steady-state condition.³⁸ Likewise, Jung *et al.* found that murine eosinophils support class switching of IgA, maintain intestinal mucus secretions, alternate in commensal intestinal microbiota and oral tolerance induction, and promote the development of Peyer's patches.³⁹ Moreover, recent studies have shown that eosinophils from the small intestinal and lamina propria regulate Th17 cells by producing IL-1 receptor antagonists and inducing the differentiation of naïve T cells into Treg cells through TGF- β 1 and retinoic acid, respectively.^{40,41}

Bone marrow and blood vessels

Eosinophils in the BM are a major source for plasma cell survival factors including a proliferation-inducing ligand (APRIL) and IL-6, and they are involved in the maintenance of plasma cells.⁴² Chu *et al.* reported that eosinophil-deficient mice reduced the number of plasma cells in the BM in both a steady-state and a state of immunization.⁴² Consecutively, they showed that the activation of BM eosinophils conditioned the expressions of APRIL, IL-6, IL-4, IL-10, and tumor necrosis factor- α (TNF- α) to support the long-term survival of plasma cells in the BM.⁴³ Together, their data suggest that eosinophils in the BM are required for the long-term maintenance of plasma cells.

In the blood stream, circulating eosinophils and other myeloid cells including macrophages and DCs produce IL-17A, an important mediator in host defense against sepsis, in an LPS-induced endotoxin shock model; this suggests a host defense role of these eosinophils.⁴⁴ By contrast, patients with HES, which is characterized by hypereosinophilia (>1500 eosinophils/ μ L for >6 months) without any known etiology (e.g., parasitic infection and allergic disorders), experience heart failure with higher rates of embolization, gastrointestinal dysfunction, central nervous system abnormalities, fever, and/or weight loss.^{45–47} Thromboembolism in hypereosinophilic heart disease seems to be induced by eosinophil cationic granule proteins such as MBP, because these toxic granules impair thrombomodulin function.⁴⁸ Moreover, Jiang *et al.* found a significant accumulation of eosinophils in the thrombus and a decrease of eosinophils in the peripheral blood of patients with acute myocardial infarction (AMI), suggesting that eosinophils play an important role in thrombosis.⁴⁹ Notably, a recent report revealed that the interaction between eosinophils and platelets promotes

atherosclerosis and stabilizes thrombosis with eosinophil extracellular traps.⁵⁰ However, definitive proof of a homeostatic role for circulating eosinophils in AMI and atherosclerosis has been lacking, suggesting that further investigations are required.

Thymus

In the thymus, eosinophils reside in the close vicinity of immature double-negative thymocytes, and the number of eosinophils is influenced by age.^{9,20,51} They decrease in number after peaking approximately two weeks after birth and only increase again at approximately 16 weeks, corresponding to the time of thymic involution^{9,20,51}; this suggests that the presence of eosinophils is likely implicated in the selection of T cells. Indeed, Throsby *et al.* demonstrated that CD11c⁺ eosinophils were preferentially recruited during class I-restricted T cell selection.⁵¹ Moreover, Kim *et al.* showed that the depletion of eosinophils resulted in the impaired clearance of apoptotic cells in the thymus.⁵² Thus, their reports suggested that the eosinophilic component of the thymic microenvironment might participate directly in the selection of T cells or aid in the scavenging of dead cells that fail negative selection and thus have an immunomodulatory role upon reaching the steady-state.

Mammary gland and uterus

Eosinophils are recruited around the growing terminal end buds (TEBs) and colocalize with macrophages during postnatal development.^{9,53} In pregnancy, which indicates the restarting of mammary development, eosinophils are again recruited to the mammary gland.⁵⁴ Gouon-Evans *et al.* demonstrated that collaboration between eotaxin-regulated eosinophils and colony-stimulating factor 1-regulated macrophages influenced TEB formation and branching morphogenesis, resulting in ductal outgrowth promotion.⁵³ Likewise, Colbert *et al.* reported that IL-5-deficient dams suppressed the development of mammary glands including TEBs, less well-developed branching of the mammary ducts, and a lower overall density of mammary gland structures; moreover, decreased litter size and weaning survival was observed in pups nursing on IL-5 deficient dams.⁵⁵

In the uterus, eosinophils are primarily located in the endometrium adjacent to the muscular layer, and the number of eosinophils ranges depending on the estrus cycle, with primarily recruited eosinophils being present.¹⁶ Although eosinophils are not essential in the normal reproduction of the uterus, which was demonstrated by the presence of normal pregnancy and parturition in eosinophil-deficient mice,¹⁶ Vicetti Miguel *et al.* reported that IL-4-producing eosinophils play an important role in endometrial maintenance and prevent Chlamydia-induced endometrial damage.⁵⁶ Moreover, Blumenthal *et al.* suggested that the abundant presence of degranulating eosinophils might be involved in tissue remodeling in human endometriosis.⁵⁷

Multiple biological behaviors of eosinophils in the pathological condition

Although eosinophilic inflammation such as AR is simply induced by the classical Th2 pathway through adaptive immunity following an allergen-specific IgE-mediated response, asthma and ECRS, which has been proposed as a new clinically diagnosed phenotype of CRSwNP,⁵⁸ have several endotypes and are recognized as heterogeneous disorders, suggesting that there is another pathway for eosinophil accumulation in the inflamed site. Indeed, recent studies have revealed that eosinophils are not only

infiltrated and activated by Th2 lymphocyte-derived IL-5 but also by ILC2-derived IL-5 stimulated by IL-1 β , IL-18, IL-25, IL-33, and TSLP signaling from epithelial cells, fibroblasts, and macrophages following stimuli (e.g., parasites, fungi, protease allergens, and viruses).^{59–61} However, the instinctive role of eosinophils in inflammation remains controversial.

Although eosinophils contribute to tissue homeostasis in steady-state conditions as described in the previous chapter, numerous studies have trended toward focusing on the contribution of eosinophils in the pathogenesis of eosinophil-associated diseases because eosinophils exert their biological effects via cytotoxic mediators such as type 2 cytokines (IL-4, IL-5, IL-9, IL-13, and IL-25), type 1 cytokines (IL-12 and interferon- γ [IFN- γ]), acute proinflammatory cytokines (TNF- α , IL-1 β , IL-6, and IL-8), chemokines (regulated by activation, normal T cell expression and secretion, and eotaxin), and lipid mediators (platelet-activating factor [PAF] and LTC4)^{2–5,62} (Table 2). By contrast, eosinophils have an anti-inflammatory effect through eosinophil-derived enzymes such as histaminase, acid phosphatase, collagenase, arylsulphatase B, phospholipase D, lysophospholipase, catalase, and nonspecific esterases.^{2–5,62} A recent study revealed that protectin D1 (PD1), a lipid mediator biosynthesized from the omega-3 fatty acid docosahexaenoic acid that is produced by eosinophils, has an anti-inflammatory effect in patients with severe asthma.⁶³ Moreover, eosinophils produce both type 2 and type 1 cytokines, suggesting their role in Th1 and Th2 balance modulation.^{64,65} Woerly *et al.* reported that signaling through CD28 receptors expressed in the eosinophils of patients with hypereosinophilic induced the secretion of Th1 cytokines such as IL-2 and IFN- γ , whereas there was no secretion of IL-4, IL-5, or IL-10.⁶⁴ Likewise, we reported that intra-tracheally instilled eosinophils following Th2 activation also resulted in an increase of IFN- γ derived from eosinophils, accompanied by airway hyperresponsiveness (AHR), and lung inflammation in mice.⁶⁶ Additionally, Takeda *et al.* showed that eosinophils contribute to the resolution of AHR and inflammation by producing IL-10 following repeated allergen challenge.⁶⁷ Collectively, it seems likely that eosinophils have several different functional roles, including proinflammatory, inhibitory, and/or regulatory effects in the inflamed site.

In studies on neoplasm, eosinophils have been known to accumulate in the tumor microenvironment in response to chemotactic factors (e.g., CCL3 [MIP-1 α], CCL5 [RANTES], CCL11 [eotaxin], and CCL24 [eotaxin-2]) that are directly or indirectly delivered by tumor cells.⁶⁸ Indeed, eosinophil accumulation is commonly observed in several types of cancer such as colorectal, breast, oral, esophageal, nasopharyngeal, laryngeal, penile, bladder, and prostate cancer and in melanoma. This tumor-associated tissue eosinophilia has been linked to improved prognosis whereas it is associated with a poor prognosis in Hodgkin lymphoma and cervical carcinoma.^{68–71} This discrepancy seems to be caused by different anti-tumorigenic or protumorigenic eosinophil effects depending on the tumor microenvironment. Infiltrated eosinophils located in the tumor display anti-tumorigenic activities by exerting their cytotoxicity through the release of mediators including ECP, EDN, EPO, MBP, TNF- α , granzyme, and IL-18, and/or via direct adhesion through Ly49, 2B4, natural killer group 2, member D (NKG2D), and LFA-1 expressed by eosinophils, which correspond with major histocompatibility (MHC) class I, CD48, MHC I chain-related molecule (MIC), and intercellular adhesion molecule-1 (ICAM-1) expressed by cancer cells, respectively.⁶⁸ By contrast, eosinophils also display protumorigenic activities; for example, eosinophils release pro-angiogenic factors (e.g., VEGF-A, fibroblast growth factor (FGF-2), CXCL8/IL-8, and osteopontin) and tumor cell proliferation and survival factors (e.g., epidermal growth factor [EGF] and transforming growth factor β

[TGF- β]) and induce matrix remodeling by secreting matrix metalloproteinases (e.g., matrix metalloproteinase [MMP2] and MMP9) that can facilitate metastatic seeding.^{68–71} Taken together, these conflicting multiple biological functions of eosinophils in the pathological condition appear to be spatiotemporally altered depending on the milieu.

Subpopulations of eosinophils classified by their features in the peripheral blood and tissue

Normodense and hypodense eosinophils

Historically, there are two distinctly different types of eosinophils: normodense and hypodense eosinophils, which are indicated by normal density (specific gravity > 1.085 g/L) and lower density (specific gravity < 1.085 g/L), respectively.^{10,11} Prin *et al.* first noted these two subtypes of eosinophils by performing density gradient centrifugation of peripheral blood leukocytes from patients with hypereosinophilia and found that higher cytotoxic eosinophil abilities were observed in hypodense eosinophils.¹¹ Supporting this, Kou *et al.* reported that an increased number of hypodense eosinophils in the peripheral blood of asthmatic subjects was correlated with clinical severity and AHR, and inhaled corticosteroids significantly decreased hypodense eosinophils in patients.⁷² Similarly, an increased number of hypodense eosinophils was observed in bronchoalveolar lavage (BAL) eosinophils following an antigen-challenged site.⁷³ In patients with AD accompanied by blood eosinophilia, increased numbers of hypodense eosinophils with morphological characteristics were consistent with an activated state and an increased concentration of ECP in the serum, which is a reflector of disease severity.⁷⁴ In animals, in addition to the presence of hypodense eosinophils in the infiltrated peritoneal eosinophils following parasite infection,⁷⁵ there is also tumoricidal activity via a granzyme B-dependent mechanism.⁷⁶ However, recently, few studies focusing on hypodense eosinophils have been reported. Nevertheless, this classification is important when performing studies that isolate activated eosinophils using a gradient of specific density method.

Resident and inducible eosinophils

Murine eosinophils isolated from digested individual tissues are classified by phenotypic marker combinations such as SSC, CD45, Siglec-F, IL-5R α , CD11b, CCR3, etc. using flow cytometric analyses (detailed list in the review by Weller¹³). Recently, Mesnil *et al.* found phenotypically distinct subtypes of eosinophils in the lung tissue under steady-state and HDM-induced allergic inflammation; these subtypes could be classified as follows: rEos (Siglec-F^{int} CD62L⁺ CD101^{low}), which are IL-5-independent parenchymal cells with a ring-shaped nucleus, and iEos (Siglec-F^{high} CD62L⁺ CD101^{high}), which are IL-5-dependent peribronchial cells with a segmented nucleus.¹² This report demonstrated that rEos expressed genes implicated in the negative regulation of immune responses (e.g., Anxa1, Nedd4, Runx3, Serpinb1a, and Ldlr) by using transcriptomic profiling analyses whereas iEos highly expressed several proinflammatory genes (e.g., Slc3a2, Tlr4, C3ar1, Il13ra1, and Il6).¹² Additionally, two phenotypically distinct types of eosinophils expressed CD62L⁺ IL-3R^{low} in the nonasthmatic lungs and CD62L^{low} IL-3R^{high} cells in the sputum of patients with asthma following double positive gating with Siglec-8 and CD125.¹²

In our study on rEos and iEos following the protocol of Mesnil *et al.*, we also confirmed the presence of phenotypically distinct rEos and iEos in the lungs from naïve and OVA-induced allergic asthma mice, and these rEos and iEos were characterized by normodense and hypodense eosinophils, respectively (Fig. 3A).

Moreover, we found that human eosinophils gated by double positive with Siglec-8 and CD125 in the peripheral blood and NPs of the same patients with ECRS were normodense and hypodense eosinophils, respectively (protocol for the purification of eosinophils from NPs⁷⁷) (Fig. 3B). A similar result was observed by Miyata *et al.*⁷⁸ Together, these results indicate that the traditional classification according to morphological changes was nearly the same as the concept of rEos and iEos classified according to these surface phenotypes.

Naïve and activated eosinophils

The modulation of surface expression levels in a large variety of receptors on eosinophils depends on spatial and environmental factors. Infiltrated eosinophils into the tissue including iEos express activation markers, which are surface proteins associated with pathological conditions. As reported by Johansson and Metcalfe,^{62,79} many activation markers determined by flow cytometry are upregulated in peripheral blood eosinophils from patients with allergic disorders (Fig. 1B). In particular, increased CD11b (α_M integrin), CD18 (β_2 integrin), CD29 (β_1 integrin), and CD32 (Fc γ RII) expressions in the activation state of blood are correlated with the severity of asthma evaluated by pulmonary functions such as FEV1/FVC, PC₂₀ (provocative concentration of methacholine or histamine producing a 20% decrease in FEV1), and FENO.^{62,79–81} Moreover, the upregulation of several surface receptors reflecting activation markers including CD11b, CD11c (α_X integrin), CD13 (Aminopeptidase N), CD18, CD25 (IL-2R α), CD29, CD58, CD63 (LAMP-3), CD66e, CD67, CD108 (semaphorin 7A), CD123 (IL-3R α), HLA-DR, and TSLPR are obviously observed on eosinophils in the BAL and/or sputum, which leads to increased inflammation.^{62,79} By contrast, the expression levels of CCR3, IL-5R α , and CD62L (L-selectin) in BAL eosinophils are reduced when compared to those in blood eosinophils, suggesting the down-regulation of receptor by internalization, a process that is known to attenuate functional responses upon prolonged exposure to agonist.^{82–84}

However, detailed analyses of tissue eosinophils from human samples including nasal polyps (NPs) and lungs have not been performed because conventional protocols lead to poor numbers of purified eosinophils obtained from tissue. To better characterize eosinophils in inflamed sites, we reported on a purification protocol using a cell sorter following a density gradient technique. Then, we found that activated eosinophils from the NPs of patients with ECRS expressed CD69 whereas eosinophils from the peripheral blood of the same patients did not. Furthermore, increased CD69 expression in tissue eosinophils was correlated with clinical findings including the NP score, sinus CT findings, and measurements of pulmonary function.⁷⁷ Likewise, Miyata *et al.* established a method for isolating CD69^{hi} CCR3^{low} CXCR4⁺ Siglec-8^{int} eosinophils from the NPs of patients with ECRS and found that increased dysregulation of fatty acid metabolism was observed in NP eosinophils compared with peripheral blood eosinophils.^{78,85} Hence, to clarify the mechanisms of activation in the inflamed site and to determine a new strategy for regulating the activation of eosinophils, additional studies must focus on comparing tissue eosinophils with peripheral blood eosinophils.

MHC class II and CD3/TCR expressed eosinophils

Eosinophils, in addition to mast cells, basophils, and ILC3s, are recognized as atypical antigen-presenting cells (APCs); conversely, DCs, macrophages, and B cells are professional APCs.⁸⁶ When evaluating MHC class II expression on eosinophils, Hansel *et al.* first reported that sputum but not blood eosinophils from patients with

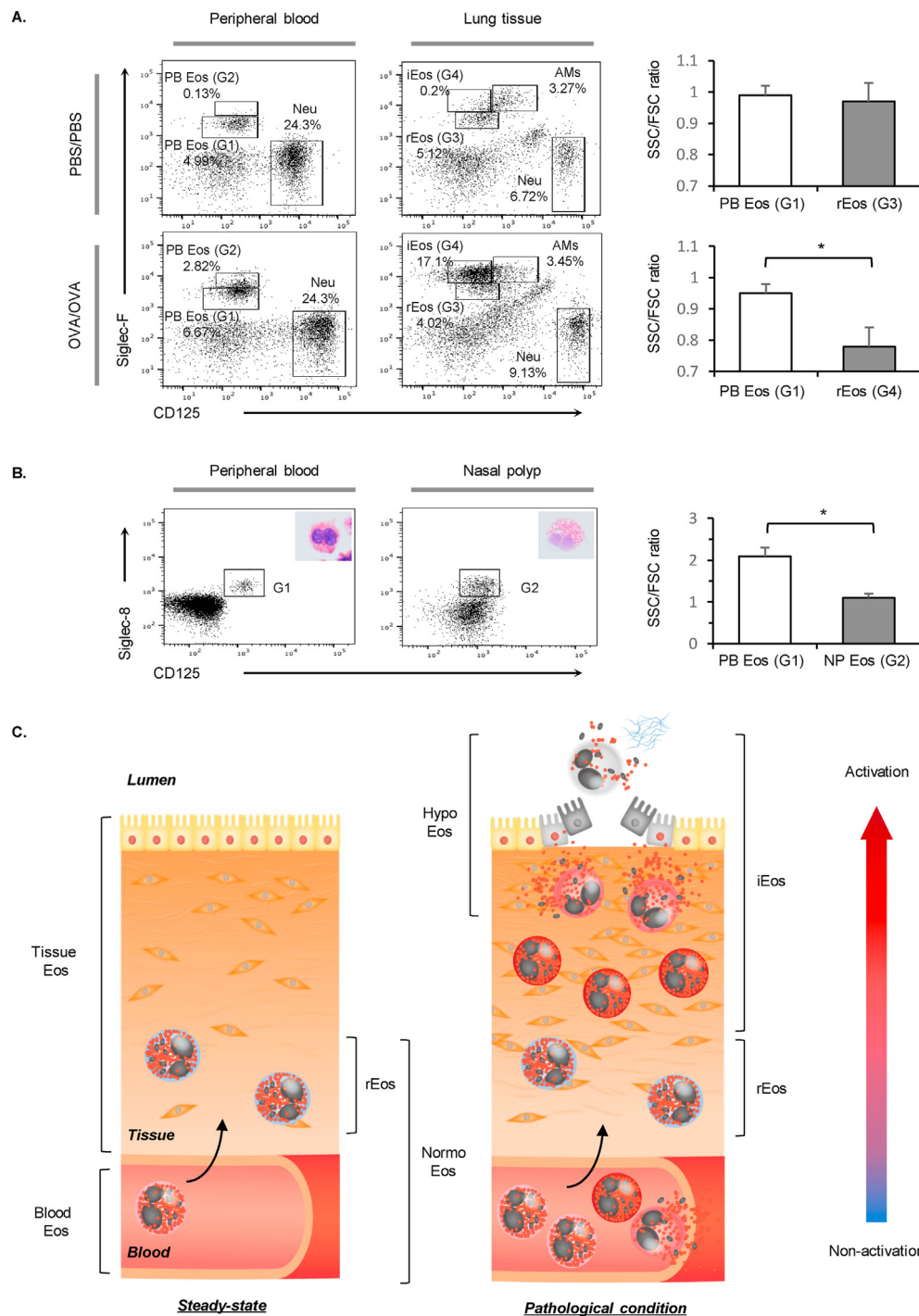


Fig. 3. Eosinophil subpopulations. The left panels in **A** and **B** indicate flow cytometric analyses in human and mouse eosinophils according to the protocol of Mesnil *et al.*,¹² respectively. The right graphs in **A** and **B** indicate the ratio SSC-A (side scatter area)/FSC-A (forward scatter area) in the individual gating. **A.** Mouse eosinophils from peripheral blood (PB) and the lungs in naïve (PBS/PBS) and asthma (OVA/OVA) models are gated by CD45⁺ Siglec-F⁺ CD125⁺. **B.** Human eosinophils from PB and naïve models are gated by CD45⁺ Siglec-8⁺ CD125⁺. The data are expressed as means $\pm$ standard errors of mean (SEMs) ($n = 3$ for each group). * Significantly different vs PB Eos, ($P < 0.01$). **C.** A schematic illustration of the subpopulation of eosinophils in the PB and tissue. Left and right panels indicate steady-state and pathological conditions, respectively. AMs, alveolar macrophages; Eos, eosinophils; hypo Eos, hypodense eosinophils; iEos, inducible eosinophils; Neu, neutrophils; norm Eos, normodense eosinophils; rEos, resident eosinophils.

asthma express MHC class II.⁸⁷ Although blood eosinophils do not express MHC class II, incubation with conditioned medium containing GM-CSF and IL-3 plus IFN- γ induced MHC class II expression on blood eosinophils.^{88,89} Likewise, in animals, the expression of MHC class II and costimulatory molecules including CD80 and CD86 proteins was observed on eosinophils in the BAL or lymph nodes of

an antigen-induced allergic model,^{90–93} and MHC class II-expressing eosinophils incubated with T cells plus specific antigens induced increased T cell proliferation and the production of cytokines from T cells.^{93,94}

Notably, several studies have shown that eosinophils express CD25 (IL-2R α is expressed on activated T cells),⁹⁵ CD4,^{96,97} and

CD28 (costimulatory molecule on T cells),⁶⁴ suggesting the existence of a subpopulation similar to T cells. Legrand *et al.* reported that human blood eosinophils expressed CD3 and $\gamma\delta$ TCR but not $\alpha\beta$ TCR following overnight culture, and $\gamma\delta$ TCR-specific agonists induced EPO, EDN, and cytokines including IFN- γ and TNF- α .⁹⁸ Together, these previous reports suggest the existence of a novel eosinophil subpopulation such as eosinophils that function similarly to APCs and T cells and are distinctly deafferented from terminally effector cells in the iEos. Further investigations focused on MHC class II expression and CD3/TCR on eosinophils will be helpful to determine the mechanism by which eosinophils contribute to antigen-induced pathology.

Future perspectives

A summarized schematic framework for the distinct subpopulations of eosinophils in the peripheral blood and tissue in steady-state and pathological conditions is shown in Figure 3C. Recently, Abdala-Valencia and colleagues reviewed tissue eosinophils classified by phenotyping and suggested dividing murine eosinophils into four sub-phenotypes including EoP, steady-state, Type 1, and Type 2. EoP includes eosinophil progenitors (CD34⁺ IL-5R α ⁺ C-KIT⁺ Sca-1⁺ TSLPR⁺ ST2⁺). Steady-state describes true tissue residents in morphogenetically quiescent tissues (Siglec-F^{med} CD101^{low} CD62⁺ IL-5R α ⁺) that display non-segmented nuclear morphology. Type 1 includes eosinophils that participate in acute inflammatory, innate defense, and transient morphogenetic contexts (Siglec-F^{med} CD11c^{low/-} CD101^{low} CD62L⁺ TLR7/8⁺ IL-5R α ⁺) that feature segmented nuclear morphology but lack vacuolarization. Type 2 typically includes iEos that play a role in the Type 2 immune response (Siglec-F^{high} CD11c^{low} CD101^{high} C5 α R⁺ 1TS⁺ IL-5R α ⁺) and feature highly segmented nuclei and the presence of vacuoles.⁹⁹ Thus, studies conducted over several years are necessary for a functional analysis of eosinophils based on phenotype classification. Investigations focused on eosinophil heterogeneity and functional plasticity in both human and murine tissues will be an exciting and emerging field that could provide further insights into the diverse roles of these cells in the context of individual organs.

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Conflict of interest

The authors have no conflict of interest to declare.

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