REVIEW ARTICLE

The emerging roles of eosinophils in mucosal homeostasis

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Eosinophils are granulocytes, typically implicated as end-stage effector cells in type-II immune responses. They are capable of producing a wide array of pre-formed molecules which render them with vast potential to influence a wide variety of processes. Nonetheless, eosinophil research has traditionally focused on their role in anti-helminthic responses and pathophysiological processes in type-II immune disorders, such as allergy and asthma, where eosinophilia is a hallmark phenotype. However, a number of key studies over the past decade have placed this restricted view of eosinophil function into question, presenting additional evidence for eosinophils as critical regulators of various homeostatic processes including immune maintenance, organ development, and tissue regeneration.

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EOSINOPHILS AT STEADY-STATE: DEVELOPMENT, RECRUITMENT, AND MAINTENANCE

Eosinophils are multi-functional granulocytes, capable of producing a wide array of molecules that are stored pre-formed in various granules and lipid bodies throughout their cytoplasm.^{1–3} Their capacity to release a large repertoire of cytokines, chemokines, lipid mediators, and granule constituents (e.g., eosinophil cationic protein, eosinophil peroxidase, major basic protein) renders these cells with vast potential to influence a wide range of processes.

Eosinophils develop in the bone marrow from GATA-binding factor 1 (GATA1) positive common granulocyte-monocyte precursor cells, which also give rise to basophils and mast cells. Differentiation into eosinophils is distinguished by the requirement for high GATA1 expression and is largely dependent on various cytokines including the eosinophil-specific factor, IL-5 (Fig. 1). These cells exit the bone marrow niche into the circulation as mature cells with a relatively short half-life (estimated to be between 3 and 18 h) where they represent <5% of total circulating leukocytes under homeostatic conditions (Fig. 1). Eosinophil chemotaxis is primarily dependent on surface expression of C-C chemokine receptor 3 (CCR3), a receptor for eotaxins, which regulates their migration and extravasation into all tissues (Fig. 1). Eotaxins include three family members, CCL11/eotaxin-1, CCL24/eotaxin-2, and CCL26/eotaxin3, each exhibiting distinct, albeit overlapping, patterns of tissue expression. The primary sources of eotaxins within tissues are stromal in origin, such as fibroblasts, smooth muscle cells and endothelial cells, but they can also be produced by epithelial cells.^{4–7}

While eosinophil recruitment and accumulation in tissues has largely been studied in the context of active type-II immune responses (i.e., helminth infection, allergic asthma), eosinophils are enriched within the thymus, adipose, and various mucosal tissues, including the intestine, uterus, lung, mammary gland, under homeostatic conditions (Table 1). In the gastrointestinal (GI)-tract (stomach, small intestine, large intestine) and thymus, homeostatic

eosinophil recruitment occurs during fetal development.^{8,9} This is in contrast to the lung, where resident eosinophils are only recruited post-natally.^{10,11} Intestinal eosinophils are recruited independent of the microbiota, with high eosinophil numbers sustained throughout life, while resident eosinophil recruitment in the lung is speculated to be influenced by the microbiota given its post-natal emergence.^{10,12} The uterus and mammary gland, whose development and remodeling is highly sensitive to sex-hormones (e.g., estrogen), have varying levels of resident eosinophils throughout life in a manner speculated to be driven (in part) by estrogen-induced eotaxin-1 production at these sites.^{13,14} Increased eosinophil recruitment to homeostatic adipose tissue requires IL-5, eotaxins and a population of CCR2-expressing cells, however the precise mechanisms by which they are recruited to healthy versus obese adipose is not known.¹⁵

In terms of eosinophil distribution, the intestine harbors the largest number of tissue-resident eosinophils in the body per total area (5–25% of total leukocytes) whereas eosinophils in the adipose tissue and the lung constitute only ~4% of stromal/vascular fraction and ~1% of total leukocytes respectively.^{10,16} In the uterus, there is an estimated 1×10^5 cells within the uterine horn but this number varies depending on the estrus cycle, with the highest eosinophil recruitment at peak.^{13,17} Similarly, eosinophil numbers in the thymus and mammary gland also fluctuate over time. While eosinophils are recruited to the cortico-medullary region (CMR) of the thymus during fetal development, peaking around 2 weeks after birth, their numbers decrease shortly thereafter and only rise again, in both the CMR and medullary region, around 16 weeks corresponding to the time of thymic involution.⁹ Eosinophils are recruited in the mammary gland around ~2.5–3 weeks of age in mice and presumably sustained only throughout the duration of mammary duct development (~8 weeks). However, they are recruited back during pregnancy when mammary development is restarted, and their presence is sustained until the weaning of pups.

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Fig. 1 Identification of tissue-resident eosinophils and their known homeostatic functions. Eosinophil development occurs in the bone marrow, arising from GATA-1+ granulocyte/macrophage progenitors (GMPs). Development into eosinophil-lineage-committed progenitors to (EoP) requires transcription factors c-EBPs and high expression of GATA1. During this transition between GMPs to EoPs, IL-33 can expand the pool of EoPs, upregulating expression of IL-5Ra. Development of EoPs into granule-mature eosinophils is facilitated by cytokines ILC2-derived IL-5 plus IL-3 and GM-CSF. Under homeostatic conditions, eosinophils exit the bone marrow into peripheral blood as mature cells identified as Siglec-F+ CCR3+ F4/80+ CD62L+ which have a relatively short half-life. Eosinophils are recruited into peripheral tissues in a manner dependent on local CCL11/eotaxin production. Tissue-resident eosinophils are primarily found in mucosal sites (i.e., GI-tract*, lung, mammary gland), but can also be found in the adipose and thymus where they are noted to contribute to various homeostatic and tissue-protective functions. The tissue microenvironment has significant influence on eosinophil characteristics demonstrated by discrepancies in surface marker expression and turnover rates.

Tissue-resident eosinophils are noted to demonstrate increased survival upon migration into peripheral tissues, where they exhibit differential turnover rates (Table 1) and surface marker expression (Fig. 1) depending on the tissue in question, indicating the importance of the tissue microenvironment/tissue-trophic cues in

fine-tuning eosinophil function. In mucosal sites, the eosinophils have prolonged survival in the intestine and uterus in contrast to the lung where their half-life is relatively shorter (Table 1). Differences in eosinophil turnover rates have been attributed to distinct expression of common γ -chain receptor, which increases

Table 1. At a glance: tissue-resident eosinophils in murine mucosal tissues.

Organ	Distribution	Recruitment	References
Intestine	Located in lamina propria Proximal small intestine (~5–10 × 10 ⁵ cells) > Distal small intestine (~3–5 × 10 ⁵ cells) > colon (~1–2 × 10 ⁵)	<ul style="list-style-type: none"> • Chemotaxis induced by CCL11/eotaxin-1 • Partial dependence on IL-5, common γ-chain • Recruitment during late embryonic development; sustained throughout adulthood • Half-life in tissue = ~6–7 days 	8, 74, 110
Lung	Located in lung parenchyma (~5 × 10 ⁵ cells)	<ul style="list-style-type: none"> • Chemotaxis induced by CCL11/eotaxin-1 • No requirement for IL-5 and common γ-chain • Post-natal recruitment; reaches adult levels 7 days after birth. • Half-life in tissue = estimated between 36 h and 8 days 	10, 11, 43, 69, 110
Mammary glands	Located in bulbous end of developing terminal end buds (TEBs) within mammary fat pads, and in cleft of TEBs pre-branching (numbers unknown)	<ul style="list-style-type: none"> • Chemotaxis induced by CCL11/eotaxin-1 • Partial-dependence on IL-5, requirements for common γ-chain unknown • Recruitment only during mammary gland development (puberty). Only reappear during pregnancy-associated mammary gland development • Half-life in tissue = unknown; only during terminal end bud (TEB) formation 	14, 62, 64
Uterus	Located in endometrium (numbers variable)	<ul style="list-style-type: none"> • Chemotaxis induced by CCL11/eotaxin-1 • Partial dependence on IL-5, common γ-chain • Cyclical recruitment with numbers peaking during estrus cycle • Half-life in tissue = ~6 days 	65, 110–112
Thymus	Located in the cortico-medullary region (numbers variable ~3 × 10 ⁵ cells)	<ul style="list-style-type: none"> • Chemotaxis induced by CCL11/eotaxin-1 • Partial-dependence on IL-5, requirements for common γ-chain unknown • Recruitment begins in the neonatal period with numbers peaking around 2 weeks after birth. Reappear during thymic involution • Half-life in tissue = ~6 days 	9, 110
Adipose tissue	Located at interstitial spaces in adipose tissue (~1 × 10 ⁵ cells/g tissue)	<ul style="list-style-type: none"> • Chemotaxis induced by CCL11/eotaxin-1 • Dependence on IL-5, requirements for common γ-chain unknown • Half-life in tissue = ~6 days 	113, 114

eosinophil survival (Table 1). This receptor is expressed in intestinal and uterine eosinophils but is absent from lung resident eosinophils. The influence of the tissue microenvironment on eosinophil characteristics demonstrates the capacity of these cells to respond to and integrate tissue-trophic cues. Given the diversity of molecules eosinophils store (and can synthesize *de novo*) and their prolonged survival in certain tissues, it would be interesting to see how and whether tissue-trophic cues in various compartments that harbor homeostatic eosinophils fine-tune the repertoire of the molecules released upon activation (e.g., degranulation) according to the demands of a given tissue. This could help explain the diversity of functions eosinophils may have in the tissues in which they reside (discussed in later sections).

In this review, we highlight recent literature aimed at investigating the functional significance of eosinophil residency within healthy tissues with a specific focus on mucosal tissues. We also discuss the novel questions these studies raise as well as their implications on our understanding of the factors contributing to mucosal homeostasis.

Factors that regulate mucosal eosinophils in health and disease IL-5 is a canonical, eosinophil-specific factor, which plays a central role in various aspects of eosinophil activation and survival in different tissues.^{18,19} IL-5 is known to prime eosinophils, lowering their threshold for degranulation in response to CCL11. Genetic deletion and antibody blockade of either CCL11 or IL-5 results in a significant decrease in eosinophils in all peripheral sites¹ (Fig. 1). It is likely that both CCL11 and IL-5 contribute to eosinophil recruitment into tissues under normal conditions, but that they can compensate for each other when one factor is absent. For the lung IL-5 mainly acts systemically to induce eosinophilia, whilst IL-4/13-induced eotaxin promotes the transmigration of eosinophils across the vascular bed into the pulmonary tissue.²⁰ Of note, blockade of both factors does not result in total ablation of eosinophil tissue recruitment, suggesting that other factors may

also influence eosinophil recruitment.²¹ Similarly to IL-5, other type-II immune cytokines such as IL-4 and IL-13, which can be sourced from Th2 cells, mast cells and basophils, can also promote eosinophil activation.^{22–24} Amongst the innate cells able to regulate eosinophil development and survival, type 2 innate lymphoid cells (ILC2s) are perhaps the most important (Fig. 1), as these cells are a rich source of type II cytokines, particularly IL-5, and represent the major tissue source of such cytokines under homeostatic conditions.^{25–27}

Apart from cytokines, various receptors expressed by eosinophils on their surface can also regulate their activation and survival.^{28–30} Siglec-F, which is expressed on the surface of eosinophils, mediates eosinophil apoptosis upon cross-linkage or ligand binding to other sialylated or sulphated glycans. Surface expression of Siglec-F correlates with eosinophil activity and is noted to be an important negative feedback mechanism limiting eosinophilia under inflammatory conditions. Eosinophils can also regulate their own survival and activation through release of autocrine signals. For example, upon contact with the extracellular matrix (ECM) protein fibronectin via VLA-4 (α 4 β 1 integrin, CD49d/CD29)-mediated adhesion, eosinophil survival is prolonged through autocrine release of GM-CSF and IL-3.^{31,32} Autocrine production of IL-5 can also be induced in response to stimulation by antibody (IgA, IgG) immune complexes.^{33,34} IL-5, GM-CSF, and IL-3 are known eosinopoietins and can function synergistically to sustain eosinophil activation and survival in peripheral tissues, and support eosinophil development in the bone marrow.^{2,35} (Fig. 1).

The epithelial-derived alarmin IL-33 is also an important regulator of eosinophil function^{36–38} and is produced both during inflammation and in homeostasis.^{36,39} In line with this, the IL-33 receptor, ST2, is expressed on the surface of eosinophils both during homeostasis (Fig. 1) and under inflammatory conditions. Eosinophilia is a major hallmark phenotype of *in vivo* IL-33 administration, which regulates eosinophil activation and expansion both directly, via ST2-signaling on eosinophils, and indirectly

via ILC2s.^{26,40–42} For example, transfer of ST2-deficient eosinophils into recipient mice subjected to allergen challenge resulted in decreased eosinophil numbers in the lung, despite normal migratory capacity.⁴³ This suggests that in vivo, tissue-derived IL-33 can act directly on eosinophils to sustain their survival. IL-33 also functions as a potent modulator of eosinophil function, driving changes in the transcriptional profile and/or promoting degranulation.^{23,44} For example, Bouffi et al.,²³ show that IL-33 stimulation of eosinophils results in the alteration of over 500 genes resulting in the upregulation of various factors such as IL-6, IL-13, CCL17, and TGF- β . In addition, IL-33 stimulation of eosinophils results in autocrine production of IL-4, which synergizes with ongoing IL-33 signaling to regulate the eosinophil transcriptome through an NF κ B-dependent mechanism. However, while the IL-4 autocrine-loop seemed to amplify IL-33 mediated activation of eosinophils, it does not account for all noted alterations, as upregulation of genes such as IL-6 and IL-13 seem to be independent of this process.²³ Eosinophil-mediated disorders such as asthma, eosinophilic esophagitis (EoE) and inflammatory bowel disease (IBD), are known to have significant input from IL-33 signaling.^{39,45,46} However, it is unclear whether dysregulation of IL-33 (and consequently—responsive cells like eosinophils) is involved in the initiation of the inflammatory response or is rather involved in its propagation.

IL-33 can also affect eosinophil function through indirect mechanisms (Fig. 1). IL-33 is a potent activator of tissue ILC2s promoting their expansion and cytokine production. ILC2s are lineage-negative, GATA3⁺ cells that are a major source of IL-13, IL-5, and amphiregulin in the early phase of type II inflammatory responses such as helminth infection, asthma and allergy.²⁵ ILC2 effector function in inflammation is achieved primarily through regulation of eosinophils (via IL-5) and goblet cell hyperplasia and mucus secretion (via IL-13). However, ILC2 are also a source of these Type II cytokines in certain tissues, like adipose, lung and intestine, under homeostatic condition.^{16,26,47} Lastly, IL-33 can induce CCL11 production in fibroblasts, which potentiates eosinophil recruitment.^{48,49}

Another epithelial-derived cytokine capable of regulating eosinophil function is IL-25, which signals through IL17RB.^{25,50,51} Similar to IL-33, IL-25 can also induce expansion of ILC2s and stimulate their production of IL-5 and 13, highlighting a degree of functional redundancy. However, unlike the IL-33 receptor ST2, it is still unclear whether or not eosinophils truly express the IL17RB receptor under homeostatic conditions. Eosinophil upregulation of surface IL17RB has been observed in a few studies, but always in the context of inflammatory responses such as allergic asthma.⁵²

CONTRIBUTIONS OF TISSUE-RESIDENT EOSINOPHILS TO TISSUE HOMEOSTASIS

Despite the long-observed phenomenon of eosinophil presence in healthy tissues at steady-state conditions, the dogmatic view of eosinophil function is that they evolved exclusively as part of an early innate immune response against parasitic helminths and additionally function in driving pathophysiological responses in type-II disorders such as asthma and EoE where eosinophilia is a common hallmark phenotype.^{53,54} The development of eosinophil-deficient mouse models (outlined in Table 2) has enabled researchers to better understand eosinophil function, particularly in inflammatory settings. As the focus of this review/commentary is on homeostatic eosinophil function we refer readers to excellent reviews for more in-depth information on the role of eosinophils in the context of eosinophil-associated disorders^{1,2,55,56} or anti-parasitic immunity.^{57,58} These models have also opened new doors and avenues to explore the functional significance of eosinophil residency in tissues under homeostasis. Given that during homeostasis the vast majority of eosinophils in the body reside in peripheral tissues, particularly

small and large intestine, adipose tissue, thymus, mammary gland and uterus, it is of interest to understand the functions of eosinophils in these diverse niches and to identify the factors that regulate their recruitment and activation state. Below we discuss relevant studies that have begun to address these fundamental questions, with a focus on mucosal tissues.

Non-mucosal tissues

Although the exact function of thymic eosinophils remains unknown, their post-natal recruitment is associated with the negative selection of MHC class I restricted T cells in the thymus⁹ and mice lacking eosinophils exhibit impaired clearance of apoptotic cells.⁵⁹ By contrast, a wealth of literature exists detailing a protective role for eosinophils within adipose tissue. The presence of eosinophils within white adipose tissue (WAT) provides protection against obesity, promotes thermogenic energy expenditure and metabolic homeostasis in mice and this is, at least in part, due to their production of IL-4, which acts to promote the alternative activation of macrophages (see reviews by^{60,61}).

Mammary gland development

Puberty hormones and growth factors trigger mammary gland development, which is characterized by the elongation and branching of the prenatal rudimentary ductal tree. Eosinophils are recruited to the postnatal mammary gland when the formation and outgrowth of terminal end buds (TEBs) occurs.¹⁴ Macrophages and eosinophils localize around the TEBs and represent the major leukocyte populations present. Their contribution to ductal outgrowth was demonstrated using sub-lethal irradiation and mice deficient in the gene encoding CSF1, a major macrophage growth factor.¹⁴ In the same study the authors used CCL11 deficient mice to show that this chemokine was the main factor responsible for eosinophil localization around the TEBs and noted reduced mammary gland branch formation. The authors conclude that macrophages regulate the elongation of the ductal tree whilst eosinophils contribute to ductal branching, perhaps by providing inhibitory signals at branching points.⁶² Intriguingly, eosinophils are again recruited to the mammary gland during pregnancy when further ductal growth occurs⁶³ and nursing of pups on IL-5 deficient dams was observed to result in a decreased survival of weanlings.⁶⁴

Endometrial maintenance

Eosinophils are also recruited to the healthy uterus during the degradation cycle of oestrus to metoestrus. Most of the eosinophils present are located in the endometrium adjacent to the muscular layer.¹³ Eosinophil recruitment and degranulation coincide with tissue-degradation and subsequent uterine repair and remodeling processes.^{65,66} Rodent eosinophils are also recruited to the cervix during gestation, increasing significantly during labor, when tissue degradative and tissue regenerative processes occur.^{67,68} However, despite this observation, eosinophil-deficient mice exhibit normal pregnancy and parturition, suggesting that the role of eosinophils in this process is redundant, or that adaptation takes place to compensate for its absence.¹³ Nonetheless, a recent study by Vicetti Miguel et al.,¹⁷ suggests that eosinophils play a critical role in endometrial maintenance during infection with *Chlamydia trachomatis*. Genital *C. trachomatis* infection promoted type II immune responses, with IL-4 signaling required for clearing infection and preventing endometrial damage.¹⁷ The use of IL-4-IRES-eGFP (4get) mice revealed eosinophils as the main source of IL-4 present during, suggesting a central role for these cells in promoting endometrial repair.¹⁷

Lung development and immunity

Eosinophils are not present in the lung of mice at birth but increase (together with ILC2s, basophils and mast cells) to reach maximal levels between postnatal day 7 and 14.⁶⁹ Their

Table 2. Eosinophil mouse models.

Eosinophil mouse model	Phenotype	Generation	Reference
dbl.GATA-1 (<i>Gata1</i> ^{tm6Sho/J})	Eosinophil absence	The transcription factor GATA-1 is expressed in erythrocytes, megakaryocytes, mast cells and eosinophils. Deletion of the palindromic (or 'double') GATA-site in the endogenous GATA-1 promoter results in impairment of GATA-1 expression in eosinophils and leads to absence of eosinophil development. The double-GATA site is not essential for GATA-1 expression in erythrocytes, megakaryocytes or mast cells making this model useful for in vivo studies of eosinophil function.	115
PHIL Tg(Epo DTA)#Nal	Eosinophil absence	An upstream sequence of EPX supports its high-level expression in eosinophil lineage-committed cells. DTA gene linked to the EPX gene results in specific eosinophil ablation without effect on other hematopoietic-derived cell generation.	116
iPHIL Inducible eosinophil-deficient knock-in mice	Eosinophil absence	Gene knock-in of human DTR into EPX locus leads to eosinophil ablation after injection of DT into mice.	117
MBP-1 ^{-/-} /EPX ^{-/-} double knockout mice	Eosinophil absence	Ablation of the genes encoding granule proteins major basic protein-1 (MBP-1) and eosinophil peroxidase (EPX) results in inhibition of eosinophilopoiesis.	118
IL-5 ^{-/-} knockout mice	Lack helminth-induced eosinophilia	The IL-5 gene was disrupted by introduction of a neomycin resistance gene (neo') leading to inhibition of IL-5 expression. IL-5 ^{-/-} mice produce normal numbers of eosinophils, however mice infected with the parasite <i>Mesocostoides corti</i> do not show increased eosinophil numbers in the blood, bone marrow, lung or peritoneal cavity.	119
IL-5 transgenic (IL-5tg)	Widespread tissue eosinophilia and extramedullary eosinophilopoiesis	Regulatory elements from the CD3delta gene was used to induce the overexpression of IL-5 in T cells. The overexpression of IL-5 leads to expansion of white blood cells (400,000 cells/mm ³) characterized by eosinophilia due to the development of extramedullary sites of eosinophilopoiesis.	120
Epx ^{tm1.1(cre)Jlee} (eoCRE)	Eosinophil-specific gene targeting	An ORF encoding Cre-recombinase protein was inserted at the start of the eosinophil peroxidase locus. This knock-in strain is able of mediating recombination of 'floxed' reporter cassettes exclusively in eosinophil-lineage-committed cells.	121

EPX eosinophil peroxidase, DTA Diptheria toxin A subunit, IL interleukin, tg transgenic, DTR Diptheria toxin receptor, DT diphtheria toxin, ORF Open Reading Frame.

recruitment follows IL-33 release in response to the 'first breath' and is closely associated with the postnatal lung 'alveolarization period' that is characterized by epithelial cell differentiation, angiogenesis and ECM remodeling.¹¹ IL-33 plays a role in promoting type II immunity and functions to maintain alveolar macrophages with a M2 phenotype that reduce pro-inflammatory responses, although they do not impact on postnatal alveolarization.^{11,69} However early IL-33 production also results in delayed anti-bacterial effector responses¹¹ and promotes sensitization towards allergens.⁶⁹ Activated eosinophils have been reported to provide protection against lethal infection with rodent pneumovirus,⁷⁰ raising the possibility that these cells may be present in the neonatal lung to provide an extra level so innate defence against respiratory viruses. In contrast to the mouse neonatal lung, the healthy adult lung contains very few resident eosinophils,^{10,69,71} yet intriguingly these cells may serve a regulatory function even at low numbers. Mesnil et al.,¹⁰ demonstrated that the adult asthmatic lung contains two populations of eosinophils, with 'homeostatic' or resident eosinophils characterized as Siglec-F^{hi}CD62L⁺CD101⁻ cells, and 'inflammatory' or recruited eosinophils as Siglec-F^{hi}CD62L⁻CD101⁺ cells. Furthermore, resident eosinophils were important for limiting Th2-responses occurring in response to house-dust mite challenge.¹⁰

Immune homeostasis and barrier integrity in the intestine Under homeostatic conditions the intestine is home to the largest pool of eosinophils in the body.^{71,72} As mentioned earlier their

presence is microbiota-independent, but requires both CCL11 production⁸ and the presence of ILC2s²⁶ (Fig. 2). Their distribution is site specific, with the proximal small intestine harboring the highest frequency (~10% of total leukocytes) in mice. Despite the high numbers of intestinal eosinophils under homeostasis, their purpose in this tissue remains largely debated. Yet, out of all mucosal sites, tissue-resident eosinophils in the intestine are arguably the most studied under homeostatic conditions. Importantly, recent studies are revealing eosinophils as critical regulators of immune homeostasis within the intestine (Fig. 2).

A key function for intestinal eosinophils during homeostasis may be the regulation of mucosal IgA (Fig. 2). IgA⁺ B cells and IgA-secreting plasma cells are very abundant in the small intestine resulting in production of vast quantities of IgA that are transferred into the intestinal lumen. Much of the IgA produced in the small intestine has specificity against intestinal microbes and homeostatic anti-microbial secretory IgA has been suggested to mediate multiple functions including immune exclusion, inhibition of bacterial motility, neutralization, modification of microbial gene expression, enhancement of antigen uptake, and/or providing a protective niche for certain microbes (see review⁷³). Given the potential importance of IgA in mucosal homeostasis, understanding the factors that regulate IgA plasma cell survival and turn-over is important—and two studies using mice deficient in eosinophils reported decreased serum, intestinal and fecal IgA levels and reduced numbers of intestinal IgA⁺ B cells and IgA-secreting plasma cells.^{74,75} One also reported dysregulated mucus

Fig. 2 Eosinophils roles in intestinal epithelial barrier and tissue remodeling. The small intestine is home to a large population of eosinophils under homeostatic conditions. Here they communicate to immune cells such as IgA⁺ plasma cells, innate lymphoid cells (ILCs) and macrophages via the release of cytokines including IL-4, IL-13, IL-5, GM-CSF, IL-1b, BAFF, TGFb, and IL-6. They are recruited via ILC2-derived IL-5 and the chemokine CCL11, and their function and/or survival may be modulated by other secreted factors including the alarmin IL-33, and tissue specific cues such as acidic pH. Eosinophils may also promote epithelial cell renewal through the release of amphiregulin—and regulate tissue remodeling by activating fibroblasts, by virtue of their ability to release metalloproteinases (MMPs) and the stromal cell growth factors, fibroblast growth factor (FGF), nerve growth factor (NGF) and vascular endothelial growth factor (VEGF).

production and an altered microbiota.⁷⁴ Eosinophils might mediate their effect on IgA producing cells through a couple different mechanisms. Chu et al.,⁷⁴ implicated eosinophil production of B cell-activating factor belonging to the TNF family (BAFF), IL-6, TGF-β, and matrix metalloproteinase 9 (MMP9), whereas Jung et al.,⁷⁵ identified an alternative pathway involving eosinophil production of IL-1β and lymphotoxins. Intestinal eosinophils can express many toll-like receptors (TLRs),⁷⁴ and thus might sense microbial signals, which could modulate their cytokine production and ultimately regulate anti-microbial IgA production. In contrast to these reports, a later study by Forman et al.,⁷⁶ found only modest reductions in IgA⁺ B cell numbers for the small intestine, and no difference for the large intestine. Eosinophils have also been implicated in regulation of intestinal T cells. Notably, separate reports detailed eosinophil-deficient mice as harboring fewer Foxp3⁺ Treg,⁷⁴ and more Th17 cells,⁷⁷ in the small intestine under steady-state conditions. Although the exact mechanism(s) as to how eosinophils impact on T cell responses remain unknown, fewer Tregs in eosinophil-deficient mice correlate with decreased TGFβ activating factors, MMP3 and MMP9,⁷⁴ and the absence of GM-CSF-induced IL-1Rα expression by eosinophils may contribute to reduced Th17 cell numbers.⁷⁷

While these studies demonstrate that eosinophils exert tissue-protective functions in the intestine, there are discrepancies regarding their impact on IgA. It is also important to note that reported studies did not always compare the phenotype of eosinophil-deficient mice to co-housed or littermate wild-type control, and microbial differences present in separately housed cohorts of mice may lead to altered immune function independently of genotype-driven changes.⁷⁸ Nevertheless, it is clear is that eosinophils are able to secrete a multitude of factors and may impact on intestinal homeostasis through the regulation of immune cells, fibroblasts and/or epithelial cells (Fig. 2). At the current time the processes outlined in Fig. 2 remain hypothetical and further studies in which environmental factors, such as the diet and microbiota, are carefully controlled will be necessary to elucidate the exact homeostatic function of eosinophils within the healthy intestine.

LIAR HYPOTHESIS REVISITED: PERSPECTIVES ON THE FUNCTIONAL SIGNIFICANCE OF MUCOSAL EOSINOPHILS

In 2010, Lee⁵³ and colleagues published a provocative hypothesis challenging the dogmatic view of eosinophils as solely evolving

for the purpose of functioning as innate immune end-stage effector cells in type II responses. The *LIAR* hypothesis, which states that eosinophils are intrinsically homeostatic cells that regulate Local Immunity And/or Remodeling/Repair, proposes an alternate view which aims to unify the seemingly paradoxical phenomenon of eosinophil accumulation in healthy tissues and their role in immune defense. Furthermore, it proposes that the mechanisms by which eosinophils propagate pathophysiological responses may be the very same mechanisms utilized in the maintenance of tissue homeostasis in healthy tissues where they reside. In this model, the key determinant of eosinophil effector functions, such as the magnitude and extent of activities, is largely dependent on the context in a given tissue microenvironment. Indeed, tissue-resident eosinophils exhibit properties distinct from mature cells newly released from the bone marrow. These findings suggest that eosinophils integrate micro-environmental cues that may shape their function and/or context-specific roles within a given tissue. However, the precise factors and mechanisms that underlie these altered states and the implications for tissue-resident eosinophil function remain unclear.

As new data expand our understanding of the functional significance of these cells in health and disease, we are constantly reminded of the complex nature and multi-functional capability of these cells—and the ideas proposed in the *LIAR* hypothesis become ever more relevant.

Addressing the role of the microenvironment in shaping eosinophil function

The pressing need for identification of micro-environmental factors regulating eosinophil function is most evident when considering the varied and sometimes contradictory findings in studies on tissue-resident eosinophils in the intestine. The discrepancies apparent between these studies likely highlight the influence of the microenvironment (particularly the microbiota) on eosinophil function. Prenatal mice have detectable eosinophils in their intestines and similar levels of eosinophils are found in the adult intestines of germ-free and colonized mice, indicating that the microbiota is not required for eosinophil recruitment to this tissue (Table 1 and ref. ⁸). However, this does not rule out a potential role for the microbiome in modulating eosinophil survival or effector function. This theoretically could occur directly via microbial-associated molecular patterns (MAMPS) or indirectly through microbial release of metabolites. The signals could also impact eosinophils directly or indirectly through microbiota regulation of other immune cells or stromal cells (i.e., epithelial cells, fibroblasts or sensory nerves), which could then produce factors that modulate eosinophil activity. In addition, feeding may influence eosinophil residency in the intestine as the number of circulating eosinophils has long been known to increase in response to ingested food,⁷⁹ and intestinal ILC2s have been shown to enhance their secretion of IL-5 and IL-13 in response to the release of vasoactive intestinal peptide (VIP) following food intake²⁶ (Fig. 2). Both ILC2s and eosinophils also respond to IL-33 which is abundantly expressed in the healthy intestine (Fig. 2), although a contribution of this cytokine to the recruitment or function of resident intestinal eosinophils has not been determined. Intriguingly, eosinophils were shown to respond to acidic conditions by virtue of their expression of GPR65,⁸⁰ implicating a possible role for this receptor in their intestinal recruitment (Fig. 2). Although we specifically discuss the intestine here, the same rules could apply to other mucosal sites such as the lung and the uterus that have their own unique composition of cellular networks and microbial communities.

It remains to be clarified whether eosinophil plasticity or heterogeneity underlies the apparent ability of these cells to contribute to mucosal tissue homeostasis during the steady state, versus their proposed pathological role in disease states such as asthma, EoE,⁸¹ and colitis.⁸¹ Tissue eosinophils may adopt different

phenotypes depending on the microenvironment in which they find themselves (plasticity). Alternatively, tissue-resident eosinophils and eosinophils newly recruited from the bloodstream in response to inflammation could exhibit different phenotypes (heterogeneity), as recently described for the lung.¹⁰ Future studies comparing eosinophil phenotype and function in healthy versus diseased mucosal tissues are needed.

A role for mucosal eosinophils in homeostatic tissue remodeling? The form and function of tissues is highly dependent on the ECM, a mesh-like network of molecules, including proteins (collagens, elastins), proteoglycans (glycosaminoglycans, heparan sulfate), and hyaluronic acid. All cells, including eosinophils, form close interactions with the ECM, which occur predominately through cell surface receptors (integrins) and biophysical stimuli (mechanical stiffness) (reviewed in refs. ^{82–84}). The ECM not only determines tissue structure but also regulates many cellular processes through determining the bio-availability of cytokines, chemokines and growth factors,^{85–87} and by directly influencing the migration and activation of cells^{83,88} hence influencing immune responses. Disruption of the homeostatic structure of the ECM results in a process of wound healing that involves four overlapping phases: the hemostatic phase, inflammatory phase, proliferation stage and remodeling stage.⁸⁹ Restoration of the ECM to its original state re-establishes homeostasis, whilst excessive deposition of connective tissue or inadequate remodeling can result in fibrosis.

Eosinophils are associated with the reinstatement of tissue homeostasis in some tissues, playing critical roles in driving regenerative responses in models of liver⁹⁰ and muscle injury⁹¹ in an IL-4 dependent manner. In keeping with the ability of eosinophils to alter muscle development, these cells are reported to promote helminth survival following primary infection with *Trichinella spiralis*.^{92,93} In this case eosinophils promote the growth of larvae within skeletal muscle,⁹² likely by enhancing nutrient metabolism within the infected cell and creating a nutrient-rich niche for the larvae.⁹³ This involves IL-4 production by eosinophils, which also functions to suppress pro-inflammatory responses in the surrounding tissue.⁹³ Of note, however, a secondary challenge of mice with the same helminth lead to the more classically observed ability of eosinophils to provide protective immunity,⁹⁴ a function that is typically associated with eosinophil activation in response to antibody binding to helminth antigens leading to adherence of the cells to the worm and the release of toxic granule proteins (reviewed in ref. ⁹⁵). Lastly, tumors are typically thought to represent a form of tissue injury and eosinophils have been noted to drive anti-tumoral responses.^{96,97} Mechanistically, the beneficial role of eosinophils under these contexts requires crosstalk and coordination with complex cellular networks encompassing other immune cells and stromal cells in a manner that blurs the lines between canonical type I and type II immune responses.

It is interesting to note that although, tissues that harbor resident eosinophils are heterogenous with regards to cellular complexity and physiology/function, they are often sites that experience homeostatic remodeling (e.g., uterus, mammary gland), mechanical stress (e.g., intestine, lung) and/or cellular turnover (e.g., thymus, intestine, uterus), as was predicated by the *LIAR* hypothesis. As mentioned above eosinophils also accumulate in certain sites (liver, muscle) in the context of injury, further highlighting their affinity to sites that experience significant cellular turnover and/or remodeling. These observations—and the commonalities between them—warrant further investigation as they could provide much needed insight into the functional significance of eosinophil tissue accumulation during homeostasis. Once within the tissue undergoing remodeling, eosinophils could integrate signals from the microenvironment and tailor their function accordingly. Whether eosinophils would then promote homeostatic or

pathophysiological remodeling would thus likely depend on (1) other cell types present, (2) external stimuli (microbiota, diet, pathogens, extent of the tissue damage), (3) physical environment (e.g., ECM, pH, mechanical stimuli) and (4) balance between inflammatory vs. anti-inflammatory signals (Fig. 2).

As mentioned earlier eosinophils are equipped with ability to interact with the ECM via expression of specific integrins (CD11c, CD11b, $\alpha 5$ integrins) and their presence during pathological inflammation (EoE, transplantation, allergy) is often associated with fibrosis¹. Under conditions of allergic inflammation airway eosinophils exhibit a hyper adhesive phenotype towards a provisional 'immature' matrix that is deposited following tissue injury⁹⁸ and their presence promotes collagen deposition and increased airway smooth muscle.⁹⁹ Eosinophils may regulate the deposition and/or remodeling of the ECM through numerous mechanisms including the direct activation of fibroblasts to secrete matrix components,¹⁰⁰ the remodeling of these components through the release of MMPs¹⁰¹ and the regulation of heparanase activity¹⁰² (Fig. 2). Eosinophils also modulate the secretion of various stromal cell growth factors including fibroblast growth factor (FGF), nerve growth factor (NGF) and vascular endothelial growth factor (VEGF)¹⁰³ (Fig. 2). Lastly, eosinophils may directly impact renewal of the epithelial barrier as human eosinophils can release amphiregulin¹⁰⁴ (Fig. 2), which is intimately involved in promoting the proliferation of epithelial cells in the intestine,¹⁰⁵ lung¹⁰⁶ and mammary gland.¹⁰⁷ In contrast to this finding, mice expressing both the IL-5 and eotaxin2 transgene's harbor increased activated eosinophils in the lung and exhibit reduced barrier function.¹⁰⁸ Thus whether eosinophils promote or reduce the integrity of epithelial layers remains unclear. Last but not least eosinophils share many functions with mast cells and these two cells often interact with each other¹⁰⁹ indicating that they may contribute in a paired manner to tissue function.

The modulation of stromal cell function (fibroblasts, neurons, epithelial cells) by eosinophils present in healthy mucosal tissues is likely (Fig. 2) but remains to be addressed experimentally (with the exception of the mammary gland where they are closely associated with ductal elongation). Nor has the possible contribution of eosinophils to homeostatic tissue remodeling been investigated. The latter point represents a particularly important point for mucosal tissues like the intestine that are constantly subject to environmental and physical stressors necessitating timely regeneration. Dysregulation of this homeostatic remodeling, or a failure to regulate the mechanical properties of the ECM during reparative responses, would be expected to impact on a variety of process cellular and tissue functions at mucosal sites.

CONCLUDING REMARKS

The evidence cited in this review supports the view that in addition to their commonly cited role in mediating tissue damage in various disorders, eosinophils participate in ongoing processes through immune regulation and tissue remodeling, with important implications for the local tissue microenvironment.

Although our appreciation of the entire spectrum of eosinophil functions has improved markedly over the last decade, we still have much to learn before the full significance of eosinophil residency within healthy mucosal tissues will be appreciated. With new tools and a broader appreciation of the possible roles of these enigmatic cells, the field is poised to move quickly and will likely deliver intriguing new insights into the functioning of homeostatic mucosal tissues. We suggest that only by deciphering the contribution of eosinophils to the complex, multi-faceted, systems that regulate homeostasis will we truly understand the etiology of the complex diseases and disorders with which eosinophils are historically associated and develop novel treatments that are targeted and efficient. This will likely require

investigators utilizing models of eosinophil deficiency to look beyond cell-cell interactions to incorporate biochemical and physiological measurements of tissue function. It will also involve determining the possible existence of distinct eosinophil subsets and the impact of distinct tissue microenvironments.

ADDITIONAL INFORMATION

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