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Eosinophils in mucosal immune responses

J Travers[1] and ME Rothenberg[1]

Eosinophils, multifunctional cells that contribute to both innate and adaptive immunity, are involved in the initiation, propagation, and resolution of immune responses, including tissue repair. They achieve this multifunctionality by expression of a diverse set of activation receptors, including those that directly recognize pathogens and opsonized targets, and by their ability to store and release preformed cytotoxic mediators that participate in host defense, to produce a variety of *de novo* pleotropic mediators and cytokines, and to interact directly and indirectly with diverse cell types, including adaptive and innate immunocytes and structural cells. Herein, we review the basic biology of eosinophils and then focus on new emerging concepts about their role in mucosal immune homeostasis, particularly maintenance of intestinal IgA. We review emerging data about their development and regulation and describe new concepts concerning mucosal eosinophilic diseases. We describe recently developed therapeutic strategies to modify eosinophil levels and function and provide collective insight about the beneficial and detrimental functions of these enigmatic cells.

INTRODUCTION

For most of its history since its discovery in the late nineteenth century, the eosinophil was considered a proinflammatory effector cell whose usefulness was limited to defense against parasites. However, knowledge gained in the past few decades has called this view into question. Eosinophils are now known to contribute to both innate and adaptive immune responses and tissue repair processes. Because they contribute to multiple phases of the immune response (initiation/polarization, effector phases, and resolution/repair), they have the potential to profoundly and diversely influence disease processes (**Figure 1**). Eosinophils are able to sense pathogens and promote innate immune responses via expression of complement receptors (CD11b), Fc receptors (FcαR, FcγRII, FcεRI, and FcεRII), and pattern recognition receptors, including multiple Toll-like receptors.[1] It should be noted that FcεRI and FcεRII are expressed in human but not murine eosinophils.[2] Upon activation, eosinophils release a variety of soluble mediators, such as cytokines, chemokines, growth factors, and bioactive lipids. Murine and human eosinophils can secrete cytokines associated with both type 1 T helper cells (Th1) (such as interferon-γ and interleukin-12 (IL-12)) and type 2 T helper cells (Th2) (such as IL-4), as well as the profibrotic cytokine transforming growth factor-β (TGF-β).[3] Eosinophil-derived IL-4 is especially important in regulating a variety of immune and metabolic processes,[4,5] including beige fat development.[6] In addition, upon activation, eosinophils also release cytotoxic, basically charged proteins, including major basic protein (MBP-1 and MBP-2), eosinophil peroxidase (EPO), eosinophil-derived neurotoxin (EDN), and eosinophil cationic protein.[7]

Eosinophils develop from eosinophil progenitor cells that are derived from hematopoietic stem cells, express CD34 (see ref. 8) and IL-5Rα, and undergo differentiation in the bone marrow upon exposure to IL-3, granulocyte-macrophage colony-stimulating factor, and IL-5. Of these three, IL-5 is unique to eosinophil differentiation and is critical for expansion of the pool of eosinophil progenitor cells.[8] IL-5Rα expression is maintained throughout all subsequent stages of eosinophil development and promotes eosinophil activation and survival, making it an attractive target for modulation of eosinophil levels. In fact, eosinophilia is enhanced in IL-5 transgenic mice[9] but is lost upon deletion of *Il5* (see ref. 10) or *Il5ra*.[11] Eosinophil development is also dependent on a complex interplay of the transcription factors GATA-binding protein 1 (GATA-1), CCAAT/enhancer-binding protein-α (C/EBPα), PU box-binding protein (PU.1), and interferon consensus sequence-binding protein (ICSBP). The critical importance of GATA-1 in eosinophil development is attested to by the fact that ΔdblGATA mice, in which the high-affinity palindromic GATA site in the *Gata1* promoter has been genetically deleted,

[1]Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA. Correspondence: ME Rothenberg (Rothenberg@cchmc.org)

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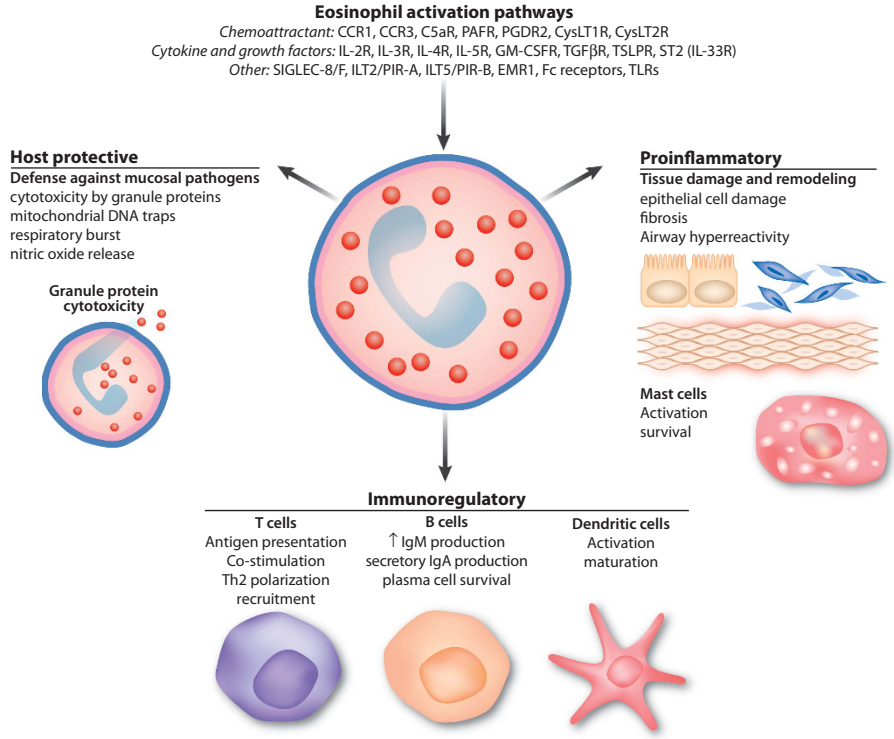

Figure 1 Eosinophil characteristics and effector functions. Eosinophil activation is mediated by a wide variety of surface receptors that respond to diverse stimuli, including cytokines, chemokines, bioactive lipids, and pathogen-associated molecular patterns. Upon activation, eosinophils promote host protection via direct effects on pathogens, immune responses by modulation of lymphocyte and dendritic cell function, and inflammation via tissue damage, remodeling, and mast cell activation. CCR, CC-chemokine receptor; CysLTR, cysteinyl leukotriene receptor; EMR, epidermal growth factor-like module containing mucin-like hormone receptor; Fc, fragment crystallizable; GM-CSFR, granulocyte-macrophage colony-stimulating factor receptor; Ig, immunoglobulin; IL, interleukin; ILT, immunoglobulin-like transcript; PAFR, platelet-activating factor receptor; PGDR, prostaglandin D2 receptor; PIR, paired immunoglobulin-like receptor; R, receptor; SIGLEC, sialic acid-binding immunoglobulin-like lectin; TGF, transforming growth factor; Th2, type 2 T helper cell; TLR, Toll-like receptor; TSLP, thymic stromal lymphopoietin.

have selective loss of eosinophils.[12] Notably, this unique double palindromic site is found within the promoter of several eosinophil-specific genes, including *Mbp*, *Epo*, and *Ccr3*.[13] Eosinophil development also depends on the interplay of the surface receptors immunoglobulin (Ig)-like transcript 2 and Ig-like transcript 5 (ILT2 and ILT5) and their murine orthologs, paired Ig-like receptors A and B (PIR-A and PIR-B), within the bone marrow. PIR-B is more highly expressed during eosinophil development and prevents PIR-A from inducing a pro-apoptotic effect.[14] Loss of PIR-B leads to apoptosis of eosinophils and prevents IL-5-mediated eosinophil expansion.[14]

Eosinophils exit the bone marrow fully differentiated and circulate within the vasculature before extravasation into tissues, where they undergo apoptosis in the absence of the proper cytokine milieu.[15] Tissue eosinophils survive for a prolonged time relative to the time that they are in circulation. For instance, in a murine adoptive transfer model, eosinophil half-life in the allergic lungs after allergen challenge was 8 days.[16] Conversely, *in vivo* studies indicate that both human[17] and murine[18] eosinophils only spend ~1 day in the bloodstream. The destination of eosinophils extravasating into the tissue is dominantly regulated by the action of CC-chemokine receptor 3 (CCR3),[3] which is relatively selective for eosinophils and is the primary receptor for the eotaxin subfamily of chemokines, CC-chemokine ligand 11 (CCL11), CCL24, and CCL26 (eotaxin-1, eotaxin-2, and eotaxin-3, respectively).[3] Eotaxin-3 is unique among the three eotaxins in that it is a nonfunctional pseudogene in mice but a functional gene in humans.[19] Although there is not a substantial overlap in the primary amino-acid sequences among the three eotaxins, their shared three-dimensional structure accounts for the common activity of these sequence-divergent proteins.[20]

REGULATION OF EOSINOPHILS IN HOMEOSTASIS

Eosinophil trafficking to mucosal tissues during homeostasis is regulated by eotaxin-1 and Th2 cytokines. Under homeostatic conditions, most eosinophils migrate to nonesophageal portions of the gastrointestinal (GI) tract under the direction of eotaxin-1 (see ref. 21) that is primarily produced by $F4/80^+CD11b^+CCR2^+Ly6C^{high}$ monocytes[22] in response to calprotectin[23] but can also be produced by intestinal epithelial cells.[24] Notably, mice deficient in CCR3 or eotaxin-1 have defective tissue homing of eosinophils to the lamina propria of the GI tract.[25] In addition, PIR-B, which decreases eosinophil responsiveness to eotaxin-1, also decreases baseline GI homing of eosinophils.[26] In addition to eotaxin-1, the Th2 cytokines IL-5 and IL-13 also are critical in sustaining GI trafficking of eosinophils during homeostasis (**Figure 2**). IL-5 promotes GI eosinophil trafficking by increasing eosinophil development

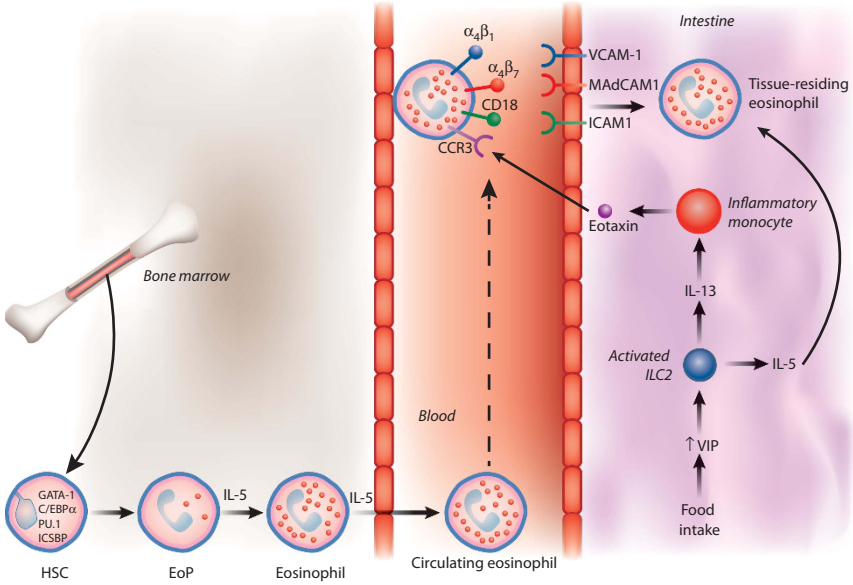

Figure 2 Homeostatic trafficking to intestine. IL-5, and to a lesser extent IL-3 and GM-CSF, promote eosinophil development in the bone marrow, trafficking into the bloodstream, and survival in the tissue. IL-13 induces eotaxin-1 release from inflammatory monocytes that causes eosinophil recruitment to the intestine via ligation of CCR3. Entry of eosinophils into the intestine is mediated by binding of $\alpha_4\beta_1$ integrin to VCAM-1, $\alpha_4\beta_7$ integrin to MAdCAM1, and CD18 family members to ICAM-1. It has been proposed that after food consumption, the neurohormone vasoactive intestinal peptide (VIP) is secreted and activates type 2 innate lymphoid cells (ILC2) within the intestine to secrete IL-5 and IL-13. BM, bone marrow; C/EBPα, CCAAT/enhancer-binding protein alpha; CCR3, CC-chemokine receptor 3; CD, cluster of differentiation; EoP, eosinophil progenitor; GATA-1, GATA-binding protein 1; GM-CSF; granulocyte-macrophage colony-stimulating factor; HSC, hematopoetic stem cell; ICAM-1, intercellular adhesion molecule 1; ICSBP, interferon consensus sequence-binding protein; IL, interleukin; MAdCAM-1, mucosal vascular addressin adhesion molecule 1; PU.1, PU box binding protein; VCAM-1, vascular cell adhesion molecule 1.

and mobilization in the bone marrow, responsiveness to eotaxin-1,[27] and survival once the eosinophils have entered the GI mucosal tissue. IL-13 increases eotaxin-1 expression.[28] Recently, the importance of type 2 innate lymphoid cells (ILC2), which are resident, IL-33-responsive cells in tissues such as the lungs and small intestine, has become appreciated. Murine ILC2 not only maintain IL-5 levels in the circulation but also link GI eosinophil levels to murine host metabolism and circadian rhythms by producing IL-5 and IL-13.[28] Vasoactive intestinal peptide, a GI neuropeptide required for maintenance of circadian rhythms, is released upon feeding and stimulates ILC2 secretion of IL-5 via ligation of the vasoactive intestinal peptide receptor type 2 (CPAC2).[28] In this manner, circadian modulation of eosinophil levels in mice is dependent on caloric intake (**Figure 2**). The relevance of these novel findings to humans is yet to be uncovered, however.

REGULATION OF EOSINOPHILS IN IMMUNE RESPONSES

Eosinophils are regulated by the epithelial-derived innate cytokines thymic stromal lymphopoietin (TSLP) and IL-33 that both directly activate eosinophils and promote their recruitment via amplification of Th2 responses. TSLP is an IL-2 family member that primes Th2 responses via activation of dendritic cells (DCs)[29] and basophils.[30] IL-33 is an IL-1 cytokine family member present in the nucleus of structural cells such as fibroblasts, epithelial cells, and endothelial cells and is released during inflammation and cellular necrosis.[31] It initiates Th2 responses by stimulating Th2 cytokine secretion (most notably IL-5 and IL-13) from ILC2.[32] In addition to promoting Th2 responses, TSLP and IL-33 act directly on eosinophils. TSLP prevents apoptosis of eosinophils by direct activation of the TSLPR present on eosinophils.[33] IL-33 potently activates murine eosinophils, including induction of marked gene expression and release of chemokines and cytokines such as IL-4.[34] IL-33 also increases the survival of human eosinophils[35] and, in a murine adoptive transfer model system, provides a survival advantage that allows for greater pulmonary trafficking.[16] This result highlights the importance of the direct effects of these innate cytokines on eosinophil function and justifies their use as potential targets to modify eosinophil function in disease states.

IMMUNOMODULATORY ROLES OF EOSINOPHILS

Eosinophils have the capacity to initiate and polarize adaptive immune responses. Eosinophils can present diverse classes of antigen, including those derived from bacteria, viruses, and parasites, to $CD4^+$ T cells.[36] Murine eosinophil expression of major histocompatibility complex class II and co-stimulatory molecules, such as CD40, CD80, and CD86, can be induced under certain conditions, although typically at lower levels than that of DCs.[37] There is evidence of antigen presentation by eosinophils in some experimental murine models[37,38] and of major histocompatibility complex class II expression by human eosinophils under certain conditions.[39] Eosinophils can also promote the initiation of adaptive immune responses by modulating DC migration, activation, and maturation through their secreted granule proteins. The granule protein EDN is a

known DC chemoattractant,[40] whereas EPO induces migration of DCs to draining lymph nodes.[41] In addition, both EDN and EPO induce DC activation and maturation,[41,42] as evidenced by (i) increased surface expression of co-stimulatory (CD80/CD86) and major histocompatibility complex molecules; (ii) release of proinflammatory soluble mediators (such as tumor necrosis factor α and IL-6); and (iii) increased CCR7 expression. Moreover, EDN- or EPO-mediated maturation of DC leads to enhanced antigen-specific Th2 responses to ovalbumin.[41,43] Finally, eosinophil deficiency protects mice from peanut food allergy by dysregulating DC activation; this activation is restored with reconstitution of either wild-type or IL-4-deficient eosinophils.[41] This requirement of eosinophils is site specific, as Th2 priming is unaffected with immunization in areas normally devoid of eosinophils such as peritoneum, skin, or rectum. These results emphasize both the potent ability that eosinophils have in the modulation of DC function and the importance of eosinophils themselves in initiating adaptive immune responses in specific mucosal tissues.

In addition to the aforementioned effects on DCs, eosinophils can also regulate the magnitude and Th2 polarization of adaptive immune responses through interactions with B and T lymphocytes. Eosinophils promote Th2 cell recruitment by inducing expression of T-cell chemoattractants like macrophage-derived chemokine (MDC/CCL22) and thymus and activation-regulated chemokine (TARC/CCL17).[44] Eosinophils also promote Th2 polarization of naive T helper cells by the release of IL-4, IL-25, and indoleamine 2,3-dioxygenase, which is selectively pro-apoptotic toward Th1 cells.[45] Eosinophil secretion of IL-4 is also important for B-cell function, as *in vivo* murine studies have demonstrated that eosinophils prime IgM production during primary immune responses to T cell-dependent antigens by secreting IL-4 that induces B-cell differentiation and thereby generates IgM-producing plasma cells.[46] In addition, eosinophils promote B-cell proliferation, survival, and antibody production upon co-culture *in vitro*.[47] However, eosinophils do not appear to be important in secondary immune responses to T cell-dependent antigens as eosinophil-deficient mice have defective IgM production in response to antigen challenge[5] without impaired generation of IgG or IgE.[48] Through these interactions with lymphocytes, eosinophils have the ability to influence both the type and magnitude of adaptive immune responses.

HOMEOSTATIC FUNCTIONAL ROLES OF EOSINOPHILS

Eosinophils regulate a variety of homeostatic processes in adipose, mucosal, and bone marrow tissues. In murine models, eosinophils present in visceral adipose tissue secrete IL-4 (see ref. 4) that causes subcutaneous white adipose tissue macrophages to polarize toward the alternatively activated phenotype[49] and express tyrosine hydroxylase.[6] The resulting increase in catecholamine production then causes the development of beige fat that ameliorates obesity-induced metabolic changes.[6] Eosinophil accumulation in the adipose tissue in these murine models is critically dependent on IL-5 and IL-13 production by resident ILC2.[50]

Eosinophils support immune homeostasis in both the gut and the bone marrow via interactions with plasma cells. Eosinophils are critical for the maintenance of plasma cell populations in the bone marrow[51] by preventing the apoptosis of bone marrow plasma cells via secretion of plasma cell survival factors like a proliferation-inducing ligand (APRIL) and IL-6,[52] of which eosinophils are the predominant source in the bone marrow.[51] It has recently been found that eosinophils sustain levels of IgA^+ plasma cells in the small intestine and are required for secretory IgA production.[53,54] One study has indicated that eosinophils mediate this effect at least in part by release of the aforementioned prosurvival factors APRIL and IL-6, whereas another has proposed that eosinophil-derived IL-1β is responsible.[54] Eosinophils could also sustain levels of IgA^+ plasma cells in the small intestine by causing increased IgA class switching, as eosinophils were found to promote IgA class switching of B cells *in vitro* through the secretion of TGF-β.[53] Regardless of the exact mechanism, eosinophil maintenance of secretory IgA production has a profound influence on gut homeostasis because of the important roles it has in various immune processes, including immune exclusion[55] and M-cell sampling of antigens.[56] That eosinophil-deficient mice have an alteration in the intestinal microbial content[53,54] substantiates this influence and also further highlights the importance that eosinophils have in maintaining mucosal immune homeostasis.

ROLE IN HOST DEFENSE

Eosinophils contribute to host defense against parasites and to a lesser extent bacteria and viruses. The antiparasitic features of eosinophils have been documented dating as far back as 1939.[57] On the basis of human correlational studies and *in vitro* assays, it was once considered almost dogmatic that eosinophils were crucial for protection against parasite infections. Both peripheral and tissue eosinophilia were noted in infected individuals. In addition, correlational studies found that parasitic infections in human increase blood levels of eosinophil-derived granule proteins[58] and that eosinophilia was found to protect from subsequent infection with *Schistosoma* species.[59] *In vitro* studies found that eosinophils can directly kill a variety of helminthic species via the action of eosinophil granule proteins[60–63] and via antibody-dependent cellular cytotoxicity.[64] In contrast to these *in vitro* findings, *in vivo* murine studies have shown that eosinophils protect against only a limited number of parasites and that eosinophils directly kill helminthic larvae[65,66] but not the adult forms that are physiologically relevant.[67] However, proper elucidation of the role of eosinophils in murine models of parasite infections is confounded by the fact that mice are often not a natural host of the parasite of interest and the possibility of compensation by other immune mechanisms. The picture is further complicated by the emerging data that eosinophils *promote* successful helminth infections. For example, eosinophils assist infection with *Trichinella spiralis* because of stunting of Th1 immunity.[68] Though eosinophils are unlikely to contribute to antihelminth immunity to the same extent as previously thought, we can

conclude that eosinophilia is a hallmark of helminthic infection and that eosinophils are likely involved in a number of immunoregulatory and/or host protective effects during these infections.

In contrast to defense against helminthes, eosinophils are not typically regarded as effectors of antibacterial or antiviral responses. In fact, eosinophil numbers typically decrease in response to bacterial infection in both humans[69] and mice.[70] However, eosinophils have been shown to promote defense in murine models against some bacteria, such as *Pseudomonas aeruginosa*.[71] Eosinophil-mediated clearance of bacteria in these models is most likely mediated by release of cytotoxic granule proteins,[72,73] release of extracellular nets of mitochondrial DNA,[74] and, to a lesser extent, phagocytosis.[75] Similar to antibacterial defense, eosinophils have been shown to be important in defense against certain viruses, notably respiratory viruses. Murine eosinophils promote viral clearance upon infection with respiratory syncytial virus[76] and parainfluenza virus.[77] This is achieved both by limiting viral replication via production of nitric oxide[76] and direct digestion of RNA viruses by the granule proteins EDN and eosinophil cationic protein, both of which have ribonuclease activity.[78] Despite the hallmark symptom being peripheral eosinophilia, eosinophils do not contribute to the enhanced disease response to respiratory syncytial virus infection after previous immunization with the respiratory syncytial virus G protein in murine models.[79] The generalizability of these findings to other pathogens and to infection in humans, who usually have coinfections with multiple pathogens, is yet to be determined.

ROLES OF EOSINOPHILS IN THE PATHOGENESIS OF MUCOSAL INFLAMMATORY DISORDERS

Asthma

Eosinophils have a critical role in asthma pathogenesis in a number of experimental murine models,[80–83] although strain-specific differences in the requirement for eosinophils have been noted.[84] The recruitment of eosinophils to the lungs is primarily mediated by eotaxin-1 and eotaxin-2.[19] A strong driver of the expression of these eotaxins is IL-13 (ref. 19), which has increased expression in patients with atopic asthma.[85–87] A newly developed system for monitoring the trafficking of adoptively transferred murine eosinophils has demonstrated that eosinophil recruitment to the lungs in response to antigen challenge requires IL-5 and IL-13Rα1 expression by the recipient and CCR3 and ST2 expression by the eosinophil,[16] highlighting the importance of eotaxins and the cytokines IL-13 and IL-33 to pulmonary eosinophil recruitment.

Upon recruitment to the lungs, eosinophils promote acute disease pathology, including airway edema and bronchoconstriction, via modulation of the function of leukocytes, including mast cells and T lymphocytes, and structural cells like epithelial cells and smooth muscle nerves. Eosinophils promote both the recruitment and function of Th2 cells in the lungs. Eosinophils are required for pulmonary recruitment of effector T cells via their secretion of IL-13,[88] CCL17, and CCL22.[44] Eosinophils are required for the function of effector T lymphocytes, as defects in producing IL-13, but not IL-4, are seen in T cells in the absence of eosinophils.[89] In addition, eosinophils promote disease pathology by promoting mast cell responses through the release of nerve growth factor, which acts in an autocrine manner on the eosinophils to induce EPO release that then enhances mast cell survival.[90] In addition, eosinophils also activate mast cells via the release of their granule proteins that induce the release of a plethora of soluble mast cell-derived mediators, including cytokines, histamine, and serotonin.[91–93] Eosinophil degranulation also further promotes disease pathology through epithelial cell damage and modulation of smooth muscle function. Though only correlative, bronchial biopsies from patients with asthma show an association between eosinophil levels and epithelial damage,[94] and eosinophil granule proteins are cytotoxic toward epithelial cells *in vitro*. In addition, MBP-1 is an antagonist of the inhibitory M2 muscarinic receptor function in airway smooth muscle nerves of guinea pigs.[95,96] Though this result has not been reproduced in humans, it is likely that eosinophils contribute to bronchoconstriction via this mechanism. On the basis of its interactions with these diverse cell types, eosinophils have an important contributory role to disease pathology.

In addition to causing tissue damage, eosinophils are key regulators of repair and tissue remodeling. Extensive studies utilizing multiple eosinophil-deficient mouse strains have implicated eosinophils in airway remodeling.[97,98] In fact, attenuation of eosinophils with anti-IL-5 treatment in patients with asthma decreases deposition of extracellular matrix components within the reticular basement membrane.[99] Eosinophils modulate the activity of fibroblasts and smooth muscle cells by the release of TGF-β that stimulates smooth muscle hyperplasia and hypertrophy and regulates the profibrotic activity of fibroblasts. Therefore, in asthma, eosinophils not only significantly cause damage to the airway but also contribute to the aberrant healing response.

These roles for eosinophils in the pathogenesis of allergic asthma have been solidified by clinical trials testing the efficacy of two different humanized, IL-5-neutralizing antibodies, mepolizumab and reslizumab. These two therapeutics are very effective in reducing peripheral blood eosinophil levels[100] and have efficacy in patients with sputum eosinophilia and persistent asthma. Reslizumab has been found to improve lung function,[101] whereas mepolizumab has been found to reduce both exacerbations[102–104] and maintenance corticosteroid doses.[105] Similarly, benralizumab, which depletes IL-5Rα$^+$ cells such as eosinophils, reduces asthma exacerbations and improves lung function in patients with steroid-resistant asthma and elevated blood eosinophil levels.[106] On the basis of these clinical trial results, it is now agreed upon that eosinophils significantly contribute to asthma, at least in the distinct disease endotype characterized by sputum eosinophilia.[107] A key question is to identify *a priori* the patients who are most likely to benefit from anti-eosinophil-directed therapies, and recent studies indicate that blood eosinophils levels of >300 cells per μl are a key determinant.[102,105,106]

Eosinophilic gastrointestinal disorders/eosinophilic esophagitis (EoE)

Eosinophilic gastrointestinal disorders are disorders that are characterized by abnormal accumulation of eosinophils in the GI tract and include eosinophilic esophagitis (EoE), eosinophilic gastritis, eosinophilic enteritis, and eosinophilic colitis. Of these, EoE is the most prevalent by a considerable margin and is the best studied. The prominent histological features of EoE include eosinophil accumulation and basal layer expansion in the epithelium and fibrosis of the lamina propria. The eosinophilic infiltrate is notable, as the esophagus is the only GI segment that is free of eosinophils under homeostatic conditions. Clinical features of this disorder include atopy, dysphagia, food impaction, and proton pump inhibitor unresponsiveness.[108] Food allergens are critical drivers of the disease, as the most effective therapies are strict elimination diets and the disease reoccurs upon food reintroduction.[109,110] Although food-specific IgE is readily detectable in EoE,[111] most patients do not have food anaphylaxis.[112] Despite the lack of IgE involvement, it has recently been proposed that food-specific IgG4 may be a key pathoetiological component.[113]

Eosinophil accumulation in the esophagus in EoE contributes to its cardinal features in most experimental murine models.[114–117] The critical driver of eosinophil recruitment to the esophagus in this disorder is IL-13 (see ref. 118) (**Figure 3**), which is derived from several sources. One source of IL-13 is Th2 cells, whose development from naive $CD4^+$ T cells is promoted by DCs under the regulation of TSLP[29] and the microRNA miR-21; miR-21 is upregulated in EoE, is primarily restricted in its expression to DCs and macrophages, and promotes Th2 differentiation by silencing transcription of IL-12p35.[119] Other sources of IL-13 include invariant natural killer T cells[120] and IL-13-secreting $FoxP3^+$ T cells.[121] The critical importance of this cytokine to the disease is highlighted by the fact that IL-13 stimulation results in an EoE-like transcriptome in *in vitro* cultures of primary esophageal epithelial cells.[118] Two critical downstream targets of IL-13 are CCL26 (eotaxin-3) and periostin. In contrast with asthma, which primarily has increased eotaxin-1 and eotaxin-2 expression, EoE features CCL26, which is the predominant eotaxin family member with increased expression[122] and is strongly induced in esophageal epithelial cells by IL-13.[118] Periostin increases eosinophil adhesion *in vitro* and likely facilitates eosinophil infiltration into the esophagus.[123] It is secreted from fibroblasts in response to IL-13 and TGF-β.[123] Because eosinophils can both secrete TGF-β directly and induce TGF-β secretion from mast cells through the release of MBP, eosinophils have the capacity to auto-amplify their own infiltration. Moreover, decreased epithelial expression of the intercellular cadherin desmoglein 1 (DSG1) by IL-13 stimulation results in increased periostin

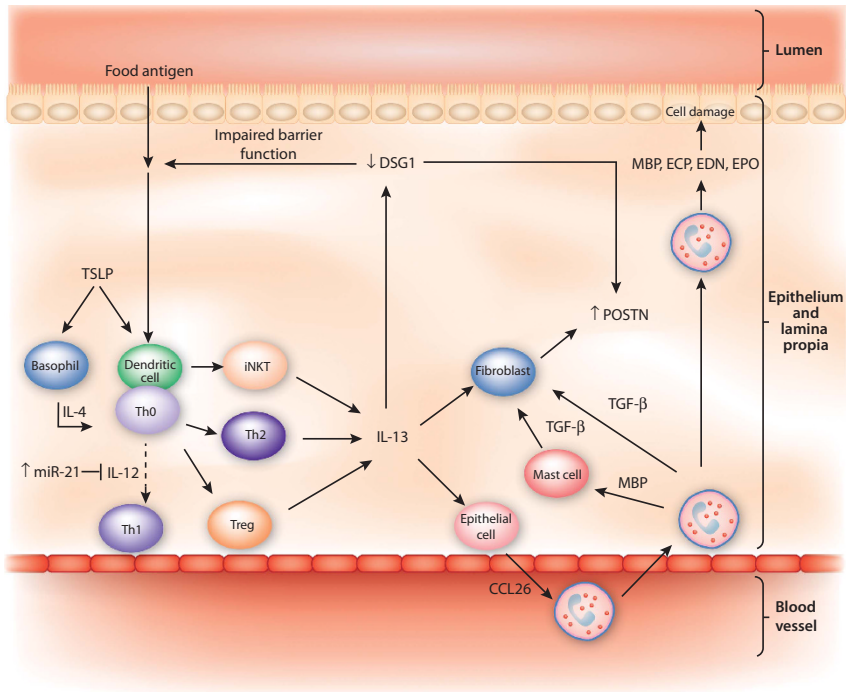

Figure 3 Pathogenesis of eosinophilic esophagitis (EoE). TSLP is released from the epithelium and activates basophils and food antigen-presenting dendritic cells to induce Th2 polarization of naive $CD4^+$ T cells. Th2 polarization is aided by miR-21 that represses Th1 polarization by degradation of IL-12. These Th2 cells, in addition to invariant natural killer T (iNKT) cells and IL-13-producing $FoxP3^+$ T cells, then secrete IL-13 that increases CCL26 and periostin (POSTN) expression and decreases desmoglein 1 (DSG1) expression in the epithelium. Decreased DSG1 level impairs barrier function that forms a propagation loop by allowing further penetration of food antigen, and also leads to increased POSTN levels. The increased CCL26 and POSTN promote eosinophil recruitment from the bloodstream. The accumulating activated eosinophils further increase POSTN expression via the release of TGF-β and also cause epithelial cell cytotoxicity. CCL, CC-chemokine ligand; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase; IL, interleukin; MBP, major basic protein; miR, microRNA; TGF, transforming growth factor; Th0, naive T helper cell; Th1, type 1 T helper cell; Th2, type 2 T helper cell; Treg, regulatory T cell; TSLP, thymic stromal lymphopoietin.

expression, as well as impaired barrier function.[124] It is interesting to note that periostin levels predict responsiveness to biological agents for asthma (such as anti-IgE and anti-IL-13),[125] highlighting the key relationship between these factors in a variety of human atopic diseases. Esophageal biopsies procured from patients with EoE typically have evidence of eosinophil degranulation,[126,127] suggesting that direct damage to esophageal epithelial cells by granule proteins may contribute to the pathogenesis of the disorder. Similar to their proposed role in asthma, eosinophils contribute to subepithelial fibrosis and dysmotility present in the EoE by secretion of TGF-β, of which they are the main source in the lamina propria.[128] TGF-β promotes fibrosis by inducing myofibroblast activation and proliferation, increased extracellular matrix synthesis, and dysmotility by inducing esophageal smooth muscle hyperplasia. Eosinophils thus have important contributions to both the damage and subsequent tissue remodeling seen in the disorder.

Despite being effective in decreasing esophageal eosinophil infiltration, the humanized, IL-5-neutralizing antibodies mepolizumab and reslizumab have only shown limited clinical improvement.[129,130] A preliminary phase II trial with the IL-13-neutralizing antibody QAX576 showed trends for clinical improvement in EoE.[131] In addition, molecular analysis of esophageal biopsies procured from participants in this trial showed that IL-13 neutralization markedly corrected the EoE transcriptome, as there was altered expression of genes in several pathways known to be dysregulated in EoE, including eosinophil recruitment, mastocytosis, tissue fibrosis, and epithelial barrier function. Though larger clinical trials still need to be performed, these collective data suggest that IL-13 is important in the development of EoE and has significant eosinophil-independent contributions to the pathogenesis of the disease.

There has been considerable progress with elucidating the genetic basis of EoE on the basis of candidate gene approaches, studies of EoE-associated Mendelian disorders, and genome-wide association studies. These interrogations have confirmed that EoE is a multifactorial disorder driven by dysregulation of several biological processes, including eosinophil recruitment, epithelial barrier function, tissue remodeling, and innate immune activation (**Figure 4**). Using a candidate gene approach, the first single-nucleotide polymorphism to be associated with EoE (rs2302009) was found within the 3′ untranslated region of *CCL26*.[122] Though the functional effect of this single-nucleotide polymorphism has not yet been determined, it likely promotes eosinophil recruitment to the esophagus by increasing *CCL26* mRNA transcription. Another candidate gene study found an association between EoE and a loss-of-function mutation of the epithelial barrier gene filaggrin (*FLG*) that results in reduced epithelial barrier function and increased allergen sensitization.[132] Genetic studies have also implicated TGF-β in the pathogenesis of the disorder. Candidate gene studies have shown that there is a genetic variant in the *TGFB* promoter, C509T, that increases transcription by creating a new binding site for the transcription factor YY1.[133] A preliminary study indicated that homozygous expression of the minor 509T allele increases the number of TGF-β-expressing cells in the esophageal lamina propria.[134] In addition, EoE is often a comorbid condition in patients with connective tissue disorders,[135] such as Marfan syndrome, Loeys–Dietz syndrome, and Ehlers–Danlos syndrome (**Figure 4**), that are associated with increased TGF-β signaling. EoE is enriched in patients with *PTEN* hamartoma tumor syndrome[136] that is caused by loss-of-function mutations in *PTEN* that result in dysregulation of cell proliferation and epithelial hyperplasia. Interestingly, loss of PTEN is known to cooperate with TGF-β in the induction of colon cancer,[137] suggesting that such interactions could also be important for the epithelial hyperproliferation seen in EoE. In many ways, TGF-β is an important contributor to disease pathogenesis in EoE. The first genome-wide association study identified a strong association at 5q22 that encodes the *TSLP* and *WDR36* genes.[138] On the basis of the known biology of TSLP and its overexpression in the esophagus of patients with EoE, it is the likely gene responsible for the genetic association. Indeed, genetic variants in the TSLP receptor *CRLF2* have also been linked with EoE using a candidate gene approach.[139] In addition to the known effects of TSLP on DCs and eosinophil function described earlier, a recent functional study has demonstrated that the 5q22 genotype affects basophil responses that have been proposed to contribute to a murine model of EoE.[140] Notably, the 5q22 locus (*TSLP*) has been linked with other atopic diseases, and hence it is unlikely to explain the tissue-specific nature of EoE (**Figure 4**). In addition to linkage to the *TSLP* gene, EoE has also been linked to the *LRRC32* gene,[141,142] which has been associated with other allergic disorders (**Figure 4**). Although not yet studied in EoE, a humanized anti-TSLP antibody (AMG 157) has been shown to lower eosinophil levels in patients with asthma.[143] A larger, recent genome-wide association study has identified a major susceptibility locus for EoE at 2p23, wherein the esophagus-specific protein calpain 14 (CAPN14) is encoded.[142] Interestingly, *CAPN14* is selectively expressed in esophageal epithelial cells, induced by IL-13, and located in an epigenetic hotspot regulated by IL-13.[142] Although the substrate(s) for CAPN14 have not yet been identified, it is notable that other calpain family members proteolytically regulate the activity of STAT6 (see ref 144) and intracellular IL-33.[145] Because of the multifactorial nature of EoE, further work is needed to expand the number of known causal variants and their mechanistic functions. To this end, the recent development of a 96-gene EoE diagnostic panel,[146] based on analysis of esophageal biopsies, provides deep information concerning the contribution of individual genes to the pathogenesis of EoE, especially on a patient-to-patient basis. This diagnostic panel, which is now commercially available and differentiates EoE from controls including gastroesophageal reflux disease, can also distinguish patients with active and inactive disease and identify glucocorticoid exposure, providing substantial clinical value in providing personalized medicine. In addition, it should prove helpful in further elucidation of the pathogenesis of mucosal eosinophilic disorders.

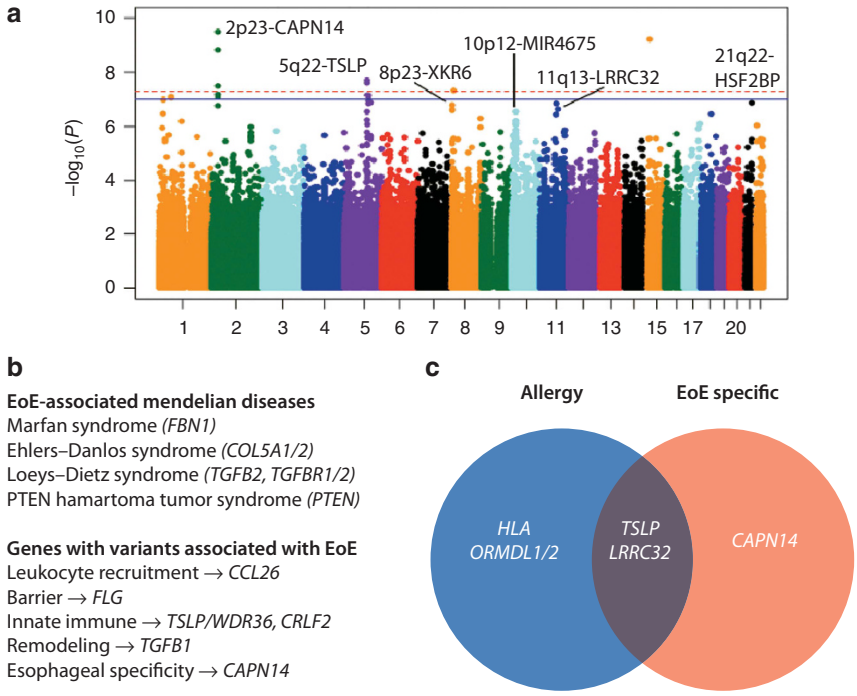

Figure 4 Genetics of eosinophilic esophagitis (EoE). (**a**) Candidate gene and genome-wide association studies have identified genetic variants in the 5q22 locus (*TSLP/WDR36*) and the 2p23 locus (*CAPN14*) as being associated with EoE susceptibility. (**b**) Other genetic risk factors include variants associated in the *CCL26*, *TGFB1*, *FLG*, and *CRLF2* genes. In addition, the PTEN hamartoma tumor syndrome (PHTS) and several inherited connective tissue disorders (CTDs) associated with TGF-β1 signaling are associated with EoE. (**c**) The EoE-associated genes do not completely overlap with genes associated with other allergic disorders, as some are specific for EoE (e.g., *CAPN14*). This figure was derived in part from a publication with permission of the copyright holder.[143] CAPN, calpain; CCL, CC-chemokine ligand; COL5A, collagen, type 5, α; CRLF, cytokine receptor-like factor; FBN1, fibrillin 1, FLG, filaggrin; HLA, human leukocyte antigen; HSF2BP, heat shock transcription factor binding protein 2; LRRC32, leucine-rich repeat-containing 32; MIR4675, microRNA 4675; ORMDL1/2, ORMDL sphingolipid biosynthesis regulator 1/2; TGF, transforming growth factor; TSLP, thymic stromal lymphopoietin; WDR, WD repeat-containing protein; XKR6, XK Kell blood group complex subunit-related family member 6.

Inflammatory bowel disease

Eosinophils are thought to contribute to the pathogenesis of inflammatory bowel disease on the basis of eosinophilia in human patient biopsy specimens and experimental murine modeling. Patients with inflammatory bowel disease often have biopsies that are notable for eosinophilia and eosinophil degranulation, especially patients with ulcerative colitis.[147] Moreover, eosinophil levels positively correlate with disease severity in these patients.[148] In addition, deficiency in MBP,[149] eotaxin-1,[24] or eosinophils themselves[150] protects against experimental murine models of colitis, although not in all cases.[151] Eosinophils also have important roles in remodeling and fibrosis that occur in ulcerative colitis, as eosinophil-depleted mice have protection from spontaneous ileitis and tissue remodeling.[152] Because levels of eotaxin-1 in the circulation and intestine are unique biomarkers for ulcerative colitis that correlate with disease severity and are useful for differentiating between active and quiescent disease,[153] a phase II trial is currently underway to investigate the efficacy of bertilimumab, an eotaxin-1-neutralizing antibody, in ulcerative colitis (ClinicalTrials.gov identifier: NCT01671956). Clinical intervention studies such as this will also help to elucidate the extent to which eosinophils contribute to the pathogenesis of inflammatory bowel disease.

PERSPECTIVE

The traditional view of eosinophils as only the "bad guys," particularly in the GI tract, has been challenged by a series of studies now implicating eosinophils as key immunoregulatory cells with a particular role in shaping the adaptive immune response (e.g., secretory IgA production) and host protection against a variety of pathogens. At the same time, there are now substantial data supporting that eosinophils contribute to the initiation, effector phase, and resolution of diverse mucosal immune responses and are thus potentially involved in a number of disorders; recent attention is now focused on eosinophilic asthma and EoE. Strong evidence for the effector role of eosinophils in mediating allergic diseases is now derived from clinical studies with anti-IL-5, anti-IL-5R, and anti-IL-13 therapeutics, at least in some subpopulations of patients with mucosal inflammatory disorders. In light of these results, IL-5 neutralization is currently being tested in eosinophilic granulomatosis with polyangiitis, an autoimmune vasculitis formerly known as Churg–Strauss syndrome with concomitant asthma and peripheral eosinophilia (ClinicalTrials.gov identifier: NCT02020889). The emerging evidence that eosinophils are pathogenic cells is prompting the development of additional eosinophil-directed therapeutics such as agents that interfere

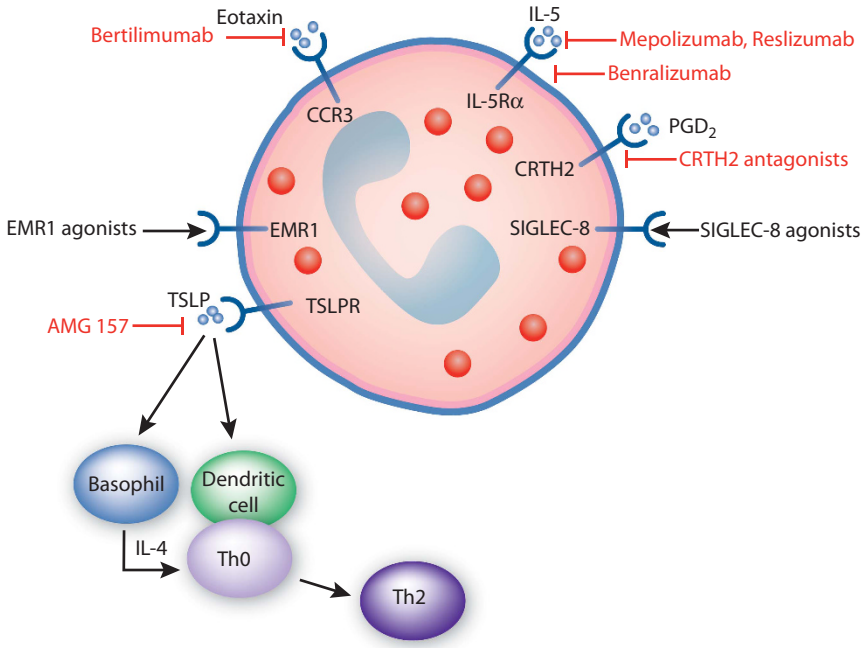

Figure 5 Targets of eosinophil-directed biological therapies. Eosinophil-directed biologic therapies function by either preventing eosinophil chemotaxis into tissues or impairing their survival upon recruitment. Eosinophil recruitment is hindered with neutralization of eotaxin by bertilimumab or blockade of CRTH2, whereas eosinophil survival is impaired with IL-5 neutralization by mepolizumab or reslizumab or IL-5Rα blockade with benralizumab. In addition, selective eosinophil ablation occurs with crosslinking of EMR1 or SIGLEC-8. Finally, neutralization of TSLP by AMG 157 impairs both eosinophil recruitment and survival, as TSLP both directly activates eosinophils and promotes Th2 differentiation and cytokine production by activating basophils and dendritic cells. CCR, CC-chemokine receptor; CRTH2, chemoattractant-homologous receptor expressed on Th2 cells; EMR, epidermal growth factor-like module containing mucin-like hormone receptor; IL, interleukin; PGD_2, prostaglandin D2; SIGLEC, sialic acid-binding immunoglobulin-like lectin; Th0, naive T helper cell; Th2, type 2 T helper cell; TSLP, thymic stromal lymphopoietin; TSLPR, thymic stromal lymphopoietin receptor.

with the eosinophil-specific surface receptors sialic acid-binding Ig-like lectin 8 (SIGLEC-8) and the epidermal growth factor-like module containing mucin-like hormone receptor 1 (EMR1).[154,155] A recent study showed that an EMR1-specific antibody induced depletion of peripheral blood eosinophils in cynomolgus monkeys, although its effects on tissue eosinophil levels are unclear.[156] The expanding number of drugs that directly target eosinophils or eosinophil-associated pathways (**Figure 5**) provide an unprecedented opportunity to advance our understanding of this enigmatic cell and improve the health of patients who have a variety of eosinophil-associated diseases.

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DISCLOSURE

M.E.R. is a consultant for Immune Pharmaceuticals, Celsus, and Receptos and has an equity interest in each and royalties from reslizumab, a drug being developed by Teva Pharmaceuticals. M.E.R. is an inventor of several patents owned by Cincinnati Children's, and a set of these patents, related to molecular diagnostics, has been licensed to Diagnovus, LLC. J.T. declares no conflict of interest.

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