REVIEW

MAYO CLINIC

Eosinophils in Health and Disease: A State-of-the-Art Review

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Abstract

Eosinophils play a homeostatic role in the body's immune responses. These cells are involved in combating some parasitic, bacterial, and viral infections and certain cancers and have pathologic roles in diseases including asthma, chronic rhinosinusitis with nasal polyps, eosinophilic gastrointestinal disorders, and hypereosinophilic syndromes. Treatment of eosinophilic diseases has traditionally been through nonspecific eosinophil attenuation by use of glucocorticoids. However, several novel biologic therapies targeting eosinophil maturation factors, such as interleukin (IL)-5 and the IL-5 receptor or IL-4/IL-13, have recently been approved for clinical use. Despite the success of biologic therapies, some patients with eosinophilic inflammatory disease may not achieve adequate symptom control, underlining the need to further investigate the contribution of patient characteristics, such as comorbidities and other processes, in driving ongoing disease activity. New research has shown that eosinophils are also involved in several homeostatic processes, including metabolism, tissue remodeling and development, neuronal regulation, epithelial and microbiome regulation, and immunoregulation, indicating that these cells may play a crucial role in metabolic regulation and organ function in healthy humans. Consequently, further investigation is needed into the homeostatic roles of eosinophils and eosinophil-mediated processes across different tissues and their varied microenvironments. Such work may provide important insights into the role of eosinophils not only under disease conditions but also in health. This narrative review synthesizes relevant publications retrieved from PubMed informed by author expertise to provide new insights into the diverse roles of eosinophils in health and disease, with particular emphasis on the implications for current and future development of eosinophil-targeted therapies.

 ■ Mayo Clin Proc. 2021;96(10):2694-2707

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Eosinophils are white blood cells that are involved in a diverse set of cellular processes in most vertebrates but are best known for their role in combating parasitic infection.[1] In diseases such as asthma, chronic rhinosinusitis with nasal polyps (CRSwNP), eosinophilic gastrointestinal (GI) disorders, and systemic hypereosinophilic diseases including eosinophilic granulomatosis with polyangiitis (EGPA) and hypereosinophilic syndrome (HES), granule proteins and chemical mediators produced by eosinophils may contribute to tissue damage, repair, remodeling, and disease persistence.[2] Interleukin (IL)-5 is one of the cytokines involved in the proliferation, maturation, activation, and recruitment of eosinophils.[3] Two other cytokines involved in type 2 (T2) immune responses characterized by eosinophilia are IL-4 and IL-13, which are also implicated in tissue eosinophil recruitment.[4] Consequently, these cytokines are targets for therapeutic interventions.

It is now recognized that eosinophils contribute to healthy homeostasis. However, knowledge gaps remain, including the need

for further characterization of eosinophil subtypes, the identification of new markers of eosinophil activation, and objective measures of clinically meaningful effects on eosinophil-mediated disease outcomes. Many findings in animal models also require validation in humans.

The basic biology of eosinophils has been reviewed extensively elsewhere.[5,6] This narrative review, employing a selective search of the literature using PubMed for articles published by December 1, 2020, summarizes findings of a literature search informed by author expert opinion, with a focus on the current understanding of the roles of eosinophils in health and disease. In addition, research priorities that may aid in the development and refinement of therapeutics for eosinophilic inflammatory diseases are highlighted.

ARTICLE HIGHLIGHTS

- Eosinophils are involved in a diverse range of processes. Human data suggest that eosinophils aid in body homeostasis; contribute to defense against parasitic, bacterial, and viral infections; are involved in cancer immunology; and have a pathologic role in eosinophilic diseases.
- Eosinophil-targeted therapies have been approved for the treatment of eosinophilic diseases, although there is a need to better understand their range of impact, factors that influence this, and their long-term safety.
- Eosinophils represent a significant therapeutic target, but unanswered questions concerning eosinophil biology still remain.

THE ROLE OF EOSINOPHILS IN HEALTH

Eosinophil Membrane Receptors and Granule Proteins

Eosinophils have receptors for a range of cytokines, chemokines, and adhesion molecules that allow them to participate in inflammatory responses and homeostasis; Fc receptors for adaptive immune system interaction; and pattern recognition receptors for the identification of pathogens in innate immune responses (Table 1).[1,7] In response to stimuli, eosinophils may release a range of granule proteins, including major basic proteins (MBPs) 1 and 2, eosinophil cationic protein (ECP), eosinophil peroxidase (EPX), eosinophil-derived neurotoxin (EDN), cytokines, and cytosolic Charcot-Leyden crystal protein/galectin-10 (CLC/Gal-10).[6,8] Granule proteins can be released by piecemeal degranulation, in which specific granule proteins are released in cytoplasmic vesicles or by cell membrane rupturing[9]; alternatively, in the eosinophil cytolytic mode of degranulation, eosinophils release clusters of cell-free intact granules and CLC protein and generate eosinophil extracellular traps (EETs), consisting of nuclear DNA fibers.[8] Eosinophils may also form EETs with mitochondrial DNA and eosinophil granule proteins.[6]

Eosinophils and their secretory products are implicated in numerous effector functions during the immune response to pathogens (Figure 1; Table 1).[6] In vitro, eosinophil-derived MBP activates basophils, mast cells, and neutrophils, whereas EDN activates human dendritic cells.[6] CLC/Gal-10 crystals also promote T2 immune responses in mice.[10] Eosinophils and the granule proteins MBP, EPX, and ECP have traditionally been considered to have antiparasitic functions, although evidence in humans is surprisingly limited and may be dependent on the specific parasite studied and experimental model used.[1,6,11] In addition, EETs together with MBP and ECP are implicated in the clearance of bacteria, and eosinophils may also have a role in antiviral responses (discussed later).[12]

Eosinophils and their secretory products may also play other essential roles. These include metabolism, fat deposition, and glucose homeostasis; tissue remodeling and development; liver and muscle repair; neuronal regulation; epithelial and microbiome regulation; and immunoregulation, including immunologic fitness in old age (Figure 2A).[1,13-15] However, whereas eosinophils appear to be involved in a wide range of physiologic processes, the majority of these observations come from studies conducted in mice, and further investigation is required to determine whether eosinophils and specific eosinophil subtypes play similar roles in

TABLE 1. Eosinophil Products and Receptors[1,7]

Cytokine and growth factor receptors	Chemoattractant receptors	Adhesion receptors	Lipid mediator receptors	Pattern recognition receptors	FcR	Inhibitory receptors	Lipid body content	Granule content
IL-2R	CCR1	LFA1 (CD11a-CD18)	PAF-R	TLR1	FCαR	Siglec-8/Siglec-f	LTC4	Cationic proteins: MBP, ECP, EDN, EPX
IL-3Rα	CCR3	CR3 (CD11a-CD18)	DP2 PG-R (CRTH2)	TLR2	FCγRII	PIR-B	15-HETE	Cytokines: IL-2, IL-3, IL-4, IL-5, IL-6, IL-10,
IL-4R	CCR4	CR4 (CD11a-CD18)	DP1 PG-R	TLR3	FCεRII	Siglec-7	PAF	IL-12, IL-13, IFNγ, GM-CSF, TGFβ, TNFα
IL-5Rα	CCR5	VLA4 (CD49d-CD29)	EP4 PG-R	TLR4	FCεRI	CD300a		Growth factors: CCL3, CCL5, CCL7,
IL-9R	CCR6	CD44	EP2 PG-R	TLR5		CD300f		CCL8, CCL13, CXCL1, CXCL10,
IL-10R	CCR8	CD62L	LTB4R	TLR6				CXCL12 Enzymes: acid phosphatase,
IL-13R	CCR9	PSGL1	CysLT1R	TLR7				collagenase, arylsulfatase B, catalase, ECP,
IL-16R (CD4)	CXCR2	CD34	CysLT2R	TLR9				EDN, EPX nonspecific esterases
IL-17R	CXCR3		P2Y2R	TLR10				
IL-23R	CXCR4			NOD1				
IL-27R	FPR1			NOD2				
IL-31R	C3aR			RIG-1				
KIT	C5aR			RAGE				
IFNγR								
TGFβR								
GMRα								

ECP, eosinophil cationic protein (ribonuclease 3); EDN, eosinophil-derived neurotoxin (ribonuclease 2); EPX, eosinophil peroxidase; FPR, formyl peptide receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GMR, GM-CSF receptor; GPR, G protein receptor; HETE, hydroxyeicosatetraenoic acid; IFN, interferon; IL, interleukin; ILT, inhibitory leukocyte immunoglobulin-like receptors; LFA, lymphocyte function–associated antigen; LIR, leukocyte immunoglobulin-like receptors; (cys)LT, (cysteinyl) leukotriene; MBP, major basic protein; NOD, nucleotide-binding oligomerization domain-containing protein; PAF, platelet-activating factor; PG, prostaglandin; PIR, paired Ig-like receptors; PSGL, P-selectin glycoprotein ligand; RAGE, receptor for advanced glycation end products; TGF, tissue growth factor; TLR, toll-like receptor; TNF, tumor necrosis factor; Tx, thromboxane.

Mayo Clin Proc. ■ October 2021;96(10):2694-2707 ■ https://doi.org/10.1016/j.mayocp.2021.04.025
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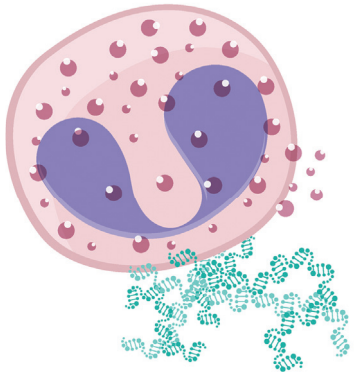

	Helminth toxicity	Antibacterial	Antiviral	Immune system	Epithelial activation and remodeling factor expression
EPX	✓	—	—	—	✓
MBP	✓	✓	—	Neutrophil, mast cell and basophil activation	✓
ECP	✓	✓	✓	—	—
EDN	—	—	✓	Dendritic cell recruitment and activation	—
CLC/gal-10	—	—	—	Th2 activation	—
EET	—	✓	—	—	—

FIGURE 1. Roles of eosinophil granule proteins and extracellular traps in innate and adaptive immune responses.[6,10] CLC/Gal-10, Charcot-Leyden crystal protein/galectin-10; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EET, eosinophil extracellular trap; EPX, eosinophil peroxidase; MBP, major basic protein.

humans. Indeed, patients treated with eosinophil-targeted therapies have so far not demonstrated any observable disruption of normal homeostatic processes.

Characterizing Eosinophil Subtypes: The Contributions of Heterogeneity and Plasticity

It has been proposed that eosinophils may be classified into different phenotypes, reflecting their potential roles in tissue processes and immune responses; those suggested include progenitor, short-lived circulatory, and steady-state/resident eosinophils and the proposed E1 and E2 subtypes.[2,3] Resident eosinophils are thought to contribute to tissue homeostasis in the lungs, adipose tissue, and GI tract,[16] whereas the E1 and E2 subtypes are both associated with inflammatory responses typically in interstitial and epithelial environments.[2] However, because macrophages and possibly other granulocytes (including eosinophils) can interconvert between different states,[17] it is currently unknown whether these groupings represent distinct eosinophil subtypes or rather a continuum of activation states. Evidence suggests that eosinophil type and function depend on the tissue microenvironment; factors associated with differential changes in eosinophil type include organ location, morphogenetic activity of the tissue (ie, steady state or developing/remodeling), location within tissue, and immune microenvironment (eg, immune neutral or predominantly T1/T17 or T2 immune signaling).[2] Further work is required to determine the drivers of eosinophil subtypes and functions and their responses to eosinophil-targeted therapies.

EOSINOPHILS IN DISEASE

Eosinophils are implicated in a range of pathologic conditions (Figure 2B), and elevated blood counts should prompt further evaluation for eosinophilic disease.[1] The upper limit of normal for blood eosinophil counts in a general population is considered to be 400 to 450 cells/μL (to convert to cells × 10^9/L, multiply by 0.001), but with adjustment for factors that cause an increase in blood eosinophil counts, the normal, adult healthy range is appreciated to be lower at 30 to 330 cells/μL (median, 120 cells/μL in men and 100 cells/μL in women).[18]

Pharmacologic Agents Targeting Eosinophils

Whereas glucocorticoids have historically been used to treat eosinophilic inflammatory diseases, several novel therapies have been introduced in recent years that target eosinophils directly or indirectly through the suppression of T2 inflammatory factors (Table 2).[1,19-38] These include therapies

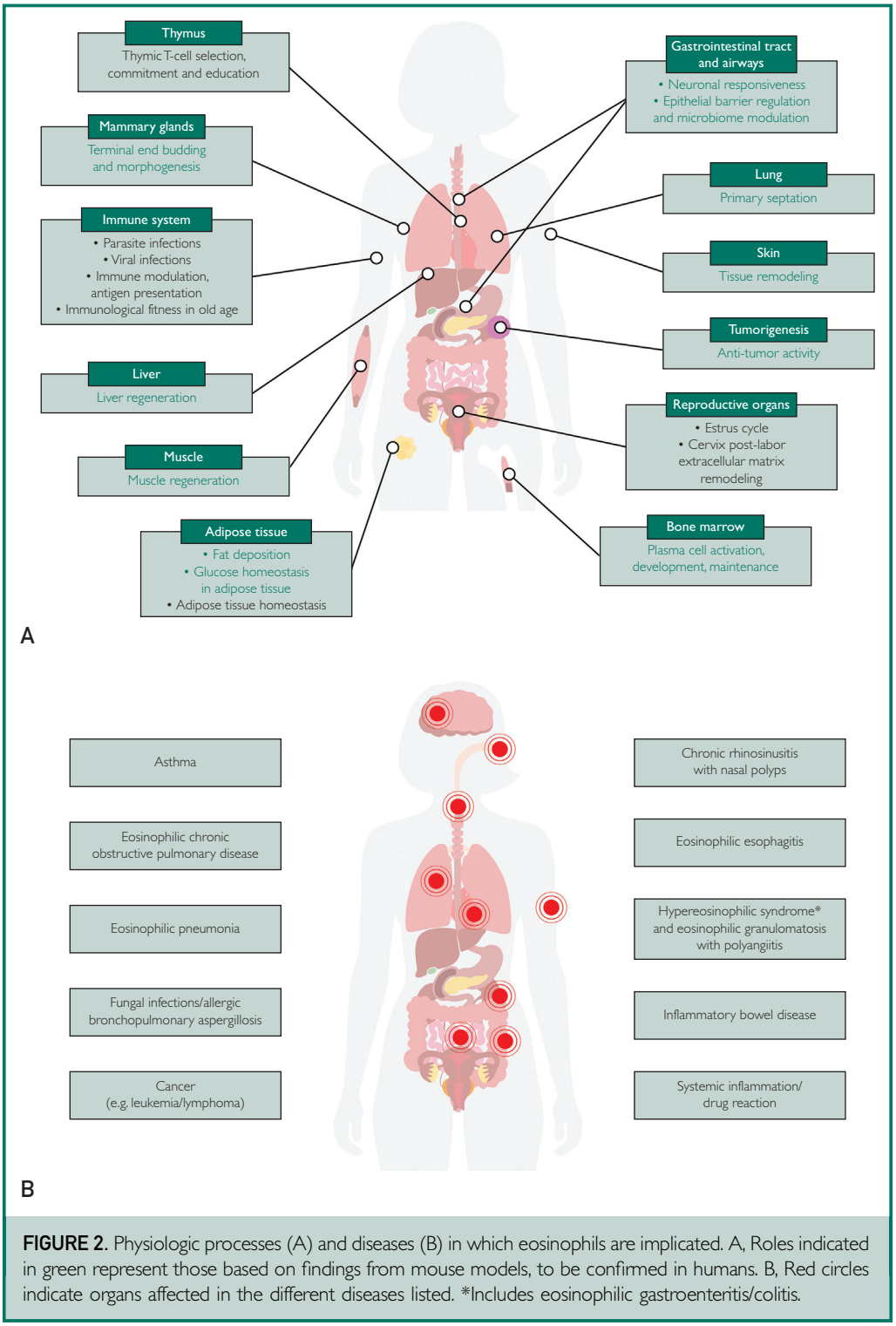

FIGURE 2. Physiologic processes (A) and diseases (B) in which eosinophils are implicated. A, Roles indicated in green represent those based on findings from mouse models, to be confirmed in humans. B, Red circles indicate organs affected in the different diseases listed. *Includes eosinophilic gastroenteritis/colitis.

against immunoglobulin (Ig) E (omalizumab; inhibiting IgE attenuates mast cell degranulation and the release of IL-5[25]), IL-5 (mepolizumab, reslizumab) and the IL-5 receptor (benralizumab; inhibiting IL-5 and its receptor attenuates eosinophil development, activation, and survival[39]), and IL-4Rα (dupilumab; inhibiting IL-4 and IL-13 attenuates B-cell

Mayo Clin Proc. ■ October 2021;96(10):2694-2707 ■ https://doi.org/10.1016/j.mayocp.2021.04.025
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TABLE 2. Eosinophil-Targeted Biologics Currently Approved and in Development[1,19-38]

Drug	Target	Approved indications	Indications in development (stage)[a]
Omalizumab	IgE	Moderate to severe asthma CRSwNP (European Union)	—
Mepolizumab	IL-5	Severe eosinophilic asthma EGPA (United States) HES (United States)	CRSwNP (phase 3) Eosinophilic COPD (phase 3) EoE (phase 2)
Reslizumab	IL-5	Severe eosinophilic asthma	HES (phase 2) EoE (phase 3)
Benralizumab	IL-5Rα	Severe eosinophilic asthma	CRSwNP (phase 3) Eosinophilic COPD (phase 3) EGPA (phase 3) HES (phase 3) EoE (phase 3) Eosinophilic gastritis/gastroenteritis (phase 2/3)
Dupilumab	IL-4Rα	Moderate to severe eosinophilic asthma CRSwNP	ABPA (phase 3) Eosinophilic COPD (phase 3) EoE (phase 2)
RPC4046	IL-13	None	EoE (phase 2)
AK002	Siglec-8	None	EoE (phase 2/3) Eosinophilic gastritis/gastroenteritis (phase 2)
Dexpramipexole	Unknown	None	Severe eosinophilic asthma (phase 2) CRSwNP (phase 2) HES (phase 3)

ABPA, allergic bronchopulmonary aspergillosis; CRSwNP, chronic rhinosinusitis with nasal polyps; COPD, chronic obstructive pulmonary disease; EGPA, eosinophilic granulomatosis with polyangiitis; EoE, eosinophilic esophagitis; HES, hypereosinophilic syndrome; IL, interleukin.

[a]Based on active studies listed on ClinicalTrials.gov.

IgE class switching, T2 cell maturation, and eosinophil recruitment and degranulation[4,25]).[19-23] Also under development are AK002, which targets siglec-8 (inhibiting mast cell degranulation, eosinophil recruitment, and inflammatory mediators[38]); RPC4046, targeting IL-13 (inhibiting IL-13 attenuates B-cell IgE class switching, T2 cell maturation, and eosinophil recruitment[4,25]); and dexapramipexole, for which the mechanism of action is unknown.[1]

Eosinophil-targeting therapies have demonstrated a favorable safety profile vs placebo across a range of eosinophilic inflammatory diseases, with no evidence of an increased incidence of infection or malignant neoplasms.[19-22,24,27] In addition, long-term results in patients with asthma treated with anti−IL-5 treatments for up to 4.5 years are consistent with the results of shorter randomized clinical trials.[40,41] The results of short-term antibody depletion of IL-5 in mice and primates as well as studies of mouse models in which eosinophils are ablated also do not appear to indicate adverse effects on health or significant differences to wild-type mice in responses to pathogens.[42-46] Efficacy data for eosinophil-targeting therapies in specific diseases are discussed in greater detail in the following.

Eosinophilic Asthma

Asthma is an inflammatory airway disease frequently characterized by airway eosinophilia.[47] Eosinophils are implicated in several pathologic processes including epithelial damage, smooth muscle hypertrophy, neural plasticity, and impaired tissue repair processes, promoting chronic airway remodeling and airflow obstruction.[3,14] In addition, elevated blood eosinophil counts are positively correlated with increased disease severity, worse disease control, and increased risk of severe exacerbations.[48,49]

Most patients achieve adequate symptom control with inhaled glucocorticoid-based

treatment.[47] However, a proportion of patients continue to experience persistent and severe disease despite adherence to inhaled glucocorticoid-containing treatment regimens, leading to frequent exacerbations requiring treatment with oral glucocorticoids.[47] In patients with severe eosinophilic asthma, mepolizumab, reslizumab, benralizumab, and dupilumab have been shown to reduce exacerbation frequency and symptom burden and to improve quality of life, with similar results for omalizumab in patients with allergic asthma.[19-23] This highlights the importance of eosinophils in the disease. In addition, T2 eosinophilic inflammation, typically identified by sputum counts of 2% and higher and/or blood counts of 150 cells/μL and higher, is an established predictor of asthma exacerbation risk and responsiveness to glucocorticoid and eosinophil-targeted therapy.[48,50,51]

Future Research. Despite the success of biologic therapies, a subset of patients with severe eosinophilic asthma continue to have uncontrolled symptoms and exacerbations.[19-22] Although this may partly relate to the presence of nonasthma comorbidities,[52] further investigation of the processes driving ongoing disease activity in these patients may help elucidate new mechanisms beyond T2 signaling in asthma. Accordingly, renewed attention should be given to how airway tissue-resident and circulating eosinophils differ and how the local airway tissue environment influences eosinophil phenotype and function. Studies have suggested that tissue-resident eosinophils differ in their responsiveness to IL-5 stimulation, surface marker expression, and effector functions and may account for the persistence of tissue eosinophilia despite treatment with anti—IL-5 agents.[16] It would also be of interest to characterize both blood and sputum eosinophils, including activation statuses, and gene array profiles in patients who are either responders or nonresponders to eosinophil-targeted treatment. A retrospective analysis (n=508) found that patients with both systemic eosinophilia (≥400 cells/μL) and airway eosinophilia (≥3%) were more likely to have worse lung functions, symptoms, and quality of life evaluations vs those with only sputum or systemic eosinophilia,[53] possibly as a result of distinct disease mechanisms. Further clarification of the mechanisms involved may lead to novel therapies to address existing unmet needs.

Finally, the development of improved clinical biomarkers to better predict a patient's response to treatment is essential. Both CLC/Gal-10 and MBP-1 in induced sputum are candidate biomarkers of eosinophilic airway inflammation,[54] and EPX has been investigated as a biomarker of eosinophil activation in induced sputum.[55] However, ECP and EDN levels in blood do not appear to have an advantage over blood eosinophil counts alone in predicting responses to treatment with anti—IL-5 agents in patients with severe eosinophilic asthma.[56]

Other Chronic Respiratory Disorders

The role of eosinophils in several other chronic respiratory disorders including CRSwNP, allergic fungal airway disease, and chronic idiopathic eosinophilic pneumonia is still under investigation. Eosinophils have also been identified as drivers of exacerbations in a subset of patients with chronic obstructive pulmonary disease (COPD),[51] and the presence of eosinophilia in COPD is a biomarker of beneficial responses to inhaled glucocorticoids.[51]

Research is now underway to assess the potential benefit of eosinophil-targeted therapy for chronic respiratory disorders (Table 2). Both omalizumab and dupilumab have been shown to have beneficial effects in patients with CRSwNP,[24,25] as has mepolizumab.[26] Similarly, a post hoc analysis of data from the MENSA trial in patients with asthma indicated that individuals with sensitization to particular fungal allergens had more pronounced reductions in the rate of exacerbations than those sensitized to aeroallergen or both fungal allergens and aeroallergens, suggesting that mepolizumab may be beneficial for patients with eosinophilic fungal airway disease.[57] In addition, a case series of 20 patients with allergic bronchopulmonary aspergillosis provided evidence that mepolizumab is associated with

reductions in the use of oral glucocorticoids and the rate of exacerbations and improvements in asthma control and quality of life.[58] In eosinophilic COPD, both mepolizumab and benralizumab have been demonstrated to reduce rates of exacerbations,[27,28] although for benralizumab only among patients with 3 or more exacerbations in the previous year or receiving triple therapy. Results from a retrospective study of mepolizumab have suggested that it may reduce relapses and lesions in patients with chronic idiopathic eosinophilic pneumonia.[59]

Future Research. A common feature in many airway diseases is the excessive production of mucus. Although CLC/Gal-10 protein functions intracellularly in eosinophil cationic ribonuclease secretion and granulogenesis,[60] recent data have implicated extracellular CLC/Gal-10 crystals, in addition to EPX, in the formation and maintenance of mucus plugs.[10,61] In addition, the concentration of CLC/Gal-10 in nasal secretions appears predictive of glucocorticoid responses in patients with CRSwNP,[62] although further determination of the utility of CLC/Gal-10 as a biomarker in clinical practice and target for treating mucus plugs is required. In the case of fungal infections, data are required on the in vitro effects of eosinophils on fungi and vice versa, especially *Aspergillus fumigatus*, and the extent to which eosinophils are protective or tissue damaging.

Hypereosinophilic Disorders

Both EGPA and HES are rare diseases characterized by tissue and blood eosinophilia and eosinophil-driven multisystem organ damage and dysfunction.[63,64] The variable nature of these diseases and their clinical overlap can make diagnosis challenging.[63,65] Most patients with EGPA[64,65] experience sinusitis, asthma, and pulmonary infiltrates, with neuropathy and vasculitis also common,[64,65] whereas HES can have broad organ involvement that produces hematologic, dermatologic, pulmonary, cardiovascular, and neurologic manifestations and, without asthma, vasculitis or antineutrophil cytoplasmic antibody positivity.[63] Subtypes of HES include myeloproliferative, lymphoproliferative, familial, and idiopathic HES.[63]

As with many eosinophilic inflammatory diseases, systemic administration of glucocorticoids has historically been the mainstay of EGPA and HES treatment.[63,64] However, studies showing the efficacy of treatment with anti—IL-5 agents in these diseases, while reducing the patient's exposure to glucocorticoid-related adverse effects, represent a significant breakthrough for patients with EGPA and HES. In the phase 3 MIRRA study, patients with EGPA receiving mepolizumab had significantly more weeks in remission and lower use of oral glucocorticoids vs placebo.[29] A post hoc analysis of the MIRRA study also concluded that 78% to 87% of patients with EGPA experienced some clinical benefit with mepolizumab with regard to time in remission, use of oral glucocorticoids, and disease relapses compared with 32% to 53% with placebo.[66] Similarly, in an open-label pilot study, benralizumab reduced EGPA exacerbations and permitted oral glucocorticoid dose reductions.[30] In patients with HES, mepolizumab decreased disease flares by 50% and reduced blood eosinophil counts vs placebo in a phase 3 study,[31] whereas a phase 2 study with benralizumab demonstrated that 90% of patients achieved a reduction of 50% or more in absolute eosinophil count after 3 months vs 30% of patients receiving placebo.[32] Additional placebo-controlled trials are also underway to investigate the effects of reslizumab and benralizumab in both diseases.[1] Overall, these results highlight the central role of eosinophils in EGPA and HES.

Future Research. The identification of clinically useful biomarkers to aid in diagnosis, prognosis, and treatment decisions in EGPA and HES is required, as is additional investigation into factors affecting their clinical presentation.[1] In particular, cardiac disease and thrombophilia can be serious complications in HES,[63] although they do not affect all patients. In addition, one form of EGPA is highly eosinophilic without clear evidence of vasculitis, antineutrophil cytoplasmic antibody negative, and difficult to distinguish

from HES, whereas another form is associated with vasculitis and has features similar to granulomatosis with polyangiitis.[67] Consequently, some patients with EGPA may benefit more from treatment with broader immunosuppressive therapy that also targets non-T2 pathways or anti—IL-5 therapy. Placebo-controlled studies of rituximab and the comparative efficacy of treatment strategies in different disease subtypes are ongoing. Therefore, insights into the molecular mechanisms underlying clinical manifestations and variability in disease presentation between patients would be valuable.

Eosinophilic Disorders of the GI Tract

Eosinophilic inflammation can also be a driving factor in a variety of eosinophilic GI disorders that can have varying disease presentations dependent on the affected area of the GI tract.[68] Such diseases include eosinophilic esophagitis (EoE), eosinophilic gastroenteritis, and eosinophilic colitis, manifestations also present in EGPA and HES.[63,64]

In 2 separate trials of mepolizumab and reslizumab in EoE, esophagus eosinophil counts were reduced but reductions in symptoms were not significantly different from those seen in patients treated with placebo.[33,34] This may be due to a range of factors including the lack of agreed regulatory end points for EoE when these trials were conducted, the variability in disease assessments between clinical trial centers, the potential importance of noneosinophil cells such as mast cells in GI disease, and the continued persistence of esophageal eosinophils at the diagnostic threshold of 15 cells or more per high-power field in approximately 50% of patients.[33,34] More recently, dupilumab, RPC4046, and AK002 have shown promise in treating EoE and reducing dysphagia and esophageal intraepithelial eosinophil counts vs placebo, although further work is needed to demonstrate the efficacy and safety of these approaches in EoE.[35-37] In eosinophilic gastritis and duodenitis, a phase 2 trial has demonstrated that AK002 vs placebo reduced GI eosinophil counts and improved symptoms.[38]

Future Research. Outstanding questions, including the role of eosinophil infiltration into GI tissues, the effect of eosinophil degranulation, and the specific pathogenic mechanisms contributing to eosinophilic GI diseases, remain. Further insights into these processes may lead to improved treatment options. In addition, some patients with eosinophil-associated GI symptoms do not have eosinophilia in the GI mucosa despite peripheral eosinophilia (unpublished data, Wardlaw, December 2020), suggesting that these patients may have a pathologic process different from that of patients with GI mucosal eosinophilia, although the reasons for this remain to be elucidated. Finally, the development of biomarkers to indicate active disease would be clinically useful.

Tumor Immunology

Although our current understanding is limited, available data suggest a pleiotropic role for eosinophils in the tumor microenvironment.[69,70] Eosinophils have been found in several tumor types with differing implications for overall survival;[69] in patients with melanoma and breast, colorectal, soft tissue, and gastric cancers, high expression of siglec-8/EPX/CLC-Gal-10 is associated with improved overall survival, whereas high expression of these biomarkers in lung and ovarian cancer is associated with diminished survival.[69] Absolute blood eosinophil counts are also positively correlated with metastatic melanoma and Hodgkin lymphoma survival.[70] Early animal studies have suggested that eosinophils may mediate tumor rejection by normalizing tumor vessels and enhancing infiltration of $CD8^+$ T cells and that eosinophil survival may be prolonged in the tumor microenvironment despite reductions in blood and bone marrow eosinophils by anti—IL-5 antibodies.[71,72] IL-5 is rarely present in tumors, and alternative eosinophil survival pathways (such as granulocyte-macrophage colony-stimulating factor) may be implicated instead.[73] In addition, mouse models of hepatocellular carcinoma and breast cancer demonstrated that eosinophil-mediated tumor control is dependent on tumor cell expression of the alarmin IL-33, whereas in lung metastasis, IL-33 promotes tumor growth by inhibiting cytolytic natural killer cells.[70,74]

Future Research. It is important to improve our understanding of the presence and function of eosinophils within different tumor types, the role of heterogeneous stromal microenvironments in driving eosinophil responses, and whether systemic eosinophil suppression with anti—IL-5 agents alters long-term cancer risk. Current evidence for mepolizumab and reslizumab suggests no increased risk of cancer when these drugs were used for up to 4.5 years.[40,41] However, as tumor types may have variable latencies, further investigation is needed. New therapies designed to selectively activate or to reprogram eosinophils to tumor-destructive phenotypes may also be valid approaches.

Antiviral Responses

Several lines of evidence suggest that eosinophils contribute to antiviral immune responses.[12] Human eosinophils are involved in viral clearance,[12,75] an effect that may be defective in patients with asthma and may contribute to virus-induced asthma exacerbations.[12,75] In addition, several eosinophil proteins, the ribonucleases EDN (ribonuclease 2) and ECP (ribonuclease 3), have antiviral properties that attenuate respiratory syncytial virus titers in vitro, and eosinophil-derived nitric oxide acts against parainfluenza virus.[12] Further indirect evidence of an antiviral role for eosinophils in humans was found in a study of patients with mild experimentally induced rhinovirus 16 infections.[76] In these patients, pretreatment with mepolizumab reduced eosinophil activation and continuing treatment resulted in an increase in rhinovirus titers alongside decreased eosinophil counts.[76] The clinical significance of increased viral titers in the setting of anti—IL-5—mediated eosinophil depletion remains unclear because these agents also reduce asthma exacerbations overall, and their use is not associated with increased severity of infections from respiratory viruses. However, evidence also suggests that eosinophils may have an indirect effect in modifying toll-like receptor 7 expression by bronchial epithelial cells, reducing innate immune responses to viral infection.[77]

Future Research. The role of eosinophils in antiviral responses and whether patients receiving antieosinophil therapies have increased susceptibility to viruses remain to be elucidated. Most recently, there has been interest in the role of eosinophils in COVID-19, although alterations in peripheral eosinophil counts are likely to be indicative of overall disease status and have not been causally implicated in viral control. In addition, asthma does not appear to be a risk factor for severe COVID-19.[78] Nonetheless, additional studies are needed to uncover the interplay of eosinophils with coronavirus and other viral infections.

THE FUTURE OF EOSINOPHIL RESEARCH

Future investigations of the basic science of eosinophils are likely to provide valuable insights to help refine diagnostic and management strategies for patients with eosinophilic inflammatory diseases. Defining specific homeostatic and pathogenic eosinophil subtypes and signals in local tissue environments in addition to organ-specific disease markers may lead to novel targeted therapies. The identification of novel biomarkers to help identify those patients most likely to respond to treatment is also a key area for future research. In addition, the use of anti—IL-5/IL-5R and IL-4/IL-13—targeted therapeutics may also provide additional insights into the roles of eosinophils in disease, with the effect of complete eosinophil depletion yet to be determined. Finally, the potential use of bispecific antibodies against multiple T2 targets may allow further disease improvements in patients with eosinophilic disease.[79]

Although animal models have provided evidence of potential homeostatic roles for eosinophils in human health, there remains a need to better understand how results from animal studies translate into humans. Currently, differences in progenitor origin, granule ultrastructure and content, surface receptors, mechanism of activation, secretion, and degranulation between eosinophils in humans and eosinophils in other species may limit the translatability of animal models, specifically mice to humans.[80] This is particularly important when examining the

potential role of eosinophil granule proteins and receptors as markers of disease as proteins such as CLC/Gal-10 are abundant in human eosinophils but absent in mice.[6] Improved animal models (eg, humanized) and other research tools may help advance understanding of the unique multifunctionality of eosinophils and their potential subtypes.

CONCLUSION

Eosinophils are involved in a diverse set of biologic processes, contributing to both normal physiologic homeostasis and disease pathology. There remains a continuing need to further investigate the homeostatic roles of eosinophils and eosinophil-mediated processes across different tissues as this is likely to provide further important insights into their actions under disease conditions. Such advances in understanding the pathophysiologic mechanisms of these diseases may enable improved diagnosis and ultimately lead to the development of novel therapeutics for eosinophilic inflammatory diseases.

ACKNOWLEDGMENTS

Editorial support (in the form of writing assistance, including preparation of the draft manuscript under the direction and guidance of the authors, collating and incorporating authors' comments for each draft, assembling tables and figures, grammatical editing, and referencing) was provided by Laura Gardner, PhD, CMPP, at Fishawack Indicia Ltd, United Kingdom, part of Fishawack Health, and was funded by GlaxoSmithKline.

Abbreviations and Acronyms: **ANCA,** antineutrophil cytoplasmic antibody; **CLC/Gal-10,** Charcot-Leyden crystal protein/galectin-10; **COPD,** chronic obstructive pulmonary disease; **CRSwNP,** chronic rhinosinusitis with nasal polyps; **ECP,** eosinophil cationic protein; **EDN,** eosinophil-derived neurotoxin; **EET,** eosinophil extracellular trap; **EGPA,** eosinophilic granulomatosis with polyangiitis; **EoE,** eosinophilic esophagitis; **EPX,** eosinophil peroxidase; **GI,** gastrointestinal; **HES,** hypereosinophilic syndrome; **Ig,** immunoglobulin; **IL,** interleukin; **MBP,** major basic protein; **T2,** type 2

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Potential Competing Interests: M.E.W. has received grants and personal fees from Novartis and Sanofi; personal fees from Regeneron, Genentech, Sentien, Restorbio, Equillium, Genzyme, and Cohero Health; grants, personal fees, and nonfinancial support from Teva, Boehringer Ingelheim, and AstraZeneca; and grants and personal fees from GlaxoSmithKline (GSK), outside the submitted work. A.Mu. is a consultant/advisory board member for GSK, Augmanity Nano Ltd, Electra-TAU, and AstraZeneca. S.J.A. is a consultant for GSK; is a co-founder, chief scientific officer, and board member of and consultant for EnteroTrack, LLC and holds equity in it; is entitled to a share of royalties from the University of Illinois at Chicago/University of Colorado in conjunction with licensing of intellectual property to EnteroTrack, LLC; and is a patent holder of a patent issued to Find Therapeutics and 3 patents licensed to EnteroTrack, LLC. M.G.D. is a consultant for GSK. D.J.J. has received personal fees from Novartis, Sanofi, Teva, Boehringer Ingelheim, GSK, and AstraZeneca. A.J.W. has received honoraria from AstraZeneca, GSK, and Pulmocide for advisory boards and research grants from Pulmocide and owns stocks/shares in GSK. S.K.D. has received research funding from Novartis, Eli Lilly, and Bristol-Myers Squibb and is a co-founder and member of the scientific advisory board for Kojin Therapeutics. S.B. reports no conflicts of interest. F.S. has received speaker's fees from Teva, AstraZeneca, Chiesi, Novartis, and GSK and consultancy fees from AstraZeneca, Chiesi, GSK, and Novartis. A.Ma. has received speaker's fees from AstraZeneca, Novartis, and GSK and honoraria for attending advisory board meetings with Sanofi, AstraZeneca, GSK, Novartis, and Chiesi. P.C. has acted as a consultant for Boehringer Ingelheim, GSK, ALK, AstraZeneca, Novartis, Teva, Chiesi, Sanofi, and SNCF; participated in advisory board meetings for Boehringer Ingelheim, GSK, Circadia, AstraZeneca, Novartis, Teva, Chiesi, and Sanofi; has received lecture fees from Boehringer Ingelheim, GSK, AstraZeneca, Novartis, Teva, Chiesi, Boston Scientific, and ALK; and has received industry-sponsored grants from Roche, Boston Scientific, Boehringer Ingelheim, Centocor, GSK, AstraZeneca, ALK, Novartis, Teva, and Chiesi. C.M.P. and P.H. are

employees of GSK and own stocks/shares. P.F.W. has acted as a consultant for GSK and has served on Data and Safety Monitoring Boards for AstraZeneca. P.A.M. has acted as a consultant for AbbVie, AstraZeneca, Biogen, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, ChemoCentryx, CSL Behring, Forbius, Genentech/Roche, Genzyme/Sanofi, GSK, InflaRx, Insmed, Janssen, Kiniksa, Kyverna, Magenta, Novartis, Pfizer, Sparrow, Takeda, and Talaris and has received research support from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, ChemoCentryx, Forbius, Genentech/Roche, Genzyme/Sanofi, GSK, and InflaRx.

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