Eosinophils and eosinophil-associated diseases: An update

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The goal of this series is to offer a survey of the latest literature for clinicians and scientists alike, providing a list of important recent advances relevant to the broad field of allergy and immunology. This particular assignment was to cover the topic of eosinophils. In an attempt to highlight major ideas, themes, trends, and advances relevant to basic and clinical aspects of eosinophil biology, a search of articles published since 2015 in the *Journal of Allergy and Clinical Immunology* and other high-impact journals was performed. Articles were then reviewed and organized, and then key findings were summarized. Given space limitations, many outstanding articles could not be included, but the hope is that what follows provides a succinct overview of recently published work that has significantly added to our knowledge of eosinophils and eosinophil-associated diseases. (J Allergy Clin Immunol 2018;141:505-17.)

Key words: *Eosinophilopoiesis, granule biogenesis, eosinophil subsets, apoptosis, adipose tissue, immunoregulation, asthma, chronic rhinosinusitis with nasal polyposis, biologicals, eosinophilic gastrointestinal disorder*

Abbreviations used

AAM:	Alternatively activated macrophage
AT:	Adipose tissue
BP:	Bullous pemphigoid
COPD:	Chronic obstructive pulmonary disease
CRS:	Chronic rhinosinusitis
CRSwNP:	Chronic rhinosinusitis with nasal polyposis
CRTH2:	Chemokine receptor homologous molecule expressed on T_H2 lymphocytes
EGID:	Eosinophilic gastrointestinal disorder
EGPA:	Eosinophilic granulomatosis with polyangiitis
EoE:	Eosinophilic esophagitis
EoP:	Eosinophil lineage–committed progenitor
EPX:	Eosinophil peroxidase
GMP:	Granulocyte-macrophage progenitor
HES:	Hypereosinophilic syndrome
IκBα:	NF-κB inhibitor α
iEos:	Inflammatory eosinophils
ILC2:	Type 2 innate lymphoid cell
MBP:	Major basic protein
NF-κB:	Nuclear factor of κ light polypeptide gene enhancer in B cells
PDGFRA:	Platelet-derived growth factor receptor α
PIR-B:	Paired immunoglobulin-like receptor B
PPI:	Proton pump inhibitor
PPI-REE:	PPI-responsive esophageal eosinophilia
Pre-GM:	Pre–granulocyte-macrophage progenitor
PVAT:	Perivascular adipose tissue
rEos:	Resident eosinophils
Siglec:	Sialic acid–binding immunoglobulin-like lectin
TH:	Tyrosine hydroxylase
WT:	Wild-type
XBP1:	X-box binding protein 1

Although definitively identified by using the aniline dye eosin by Paul Ehrlich in 1879, eosinophils had likely been described by others even before this report. This underappreciated history of the early characterization of the eosinophil was conveyed by A. B. Kay in a review entitled, "The early history of the eosinophil."[1] For example, Wharton Jones depicted "coarsely granular cells" that resembled eosinophils in a number of species, including the lamprey, frog, fowl, horse, and elephant, as well as in human subjects before production of eosin. Stacy and Raskin[2] have carried on his legacy using Wright-Giemsa staining to study eosinophils from a number of reptilian species, showing remarkable morphologic beauty and diversity among and, interestingly, even within species. The fact that evolutionary pressures over many millennia have maintained the eosinophil lineage in vertebrates is irrefutable evidence of their importance in health. Yet the role of this cell in human well-being and disease remains controversial and inexactly defined. A monumental advance in this regard occurred in 2015 and 2016 with the approval of 2 drugs, both biologicals, the mechanism of action of which is to neutralize an essential selective eosinophilopoietic cytokine, IL-5, providing a critical pharmacologic tool to begin to dissect the contribution of eosinophils to disease. The purpose of this "Fundamentals of allergy and immunology" essay is to highlight key advances in molecular, cellular, biochemical, and clinical

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aspects of eosinophil biology that provide new insight into their role in shaping homeostatic, immune, and disease-related responses.

EOSINOPHIL LINEAGE AND BASIC EOSINOPHIL BIOLOGY

Transcriptional control of development

Current data from mice indicate that eosinophils develop from populations of progenitor cells that give rise to all myeloid cells: the pre–granulocyte-macrophage progenitor (pre-GM) and the granulocyte-macrophage progenitor (GMP). However, lymphoid-primed multipotent progenitors, which give rise to lymphoid and myeloid cells but not megakaryocyte or erythroid lineages, and common myeloid progenitors, which give rise to myeloid, megakaryocyte, and erythroid lineages but not lymphoid cells, have been proposed to generate myeloid lineages through the same pre-GM population.

Drissen et al[3] used gene profiling of the pre-GM population to segregate these cells according to lineage potential. They found that expression of the transcription factor GATA-1 distinguished cells restricted to the mast cell, eosinophil, megakaryocyte, and erythroid lineages (GATA-1$^+$ pre-GMs) from those restricted to the monocyte, neutrophil, and lymphoid lineages (GATA-1$^-$ pre-GMs and GMPs, Fig 1). This result implies that there is an early developmental divergence between these families of lineages that challenges the current dogma.

To assess global transcriptomic changes that occur during homeostatic eosinophil development, Bouffi et al[4] sorted GMPs, eosinophil lineage–committed progenitors (EoPs), and mature resting eosinophils from mouse bone marrow and analyzed them through RNA sequencing. Associated with eosinophil lineage commitment (between the GMP and EoP stages) and eosinophil maturation (between the EoP and eosinophil stages) were substantial changes in 490 and 1199 genes, respectively. Included among the genes expressed by eosinophils but not GMPs were 56 transcription factors, including 2 Ikaros family members, Helios and Aiolos, that were expressed by both EoPs and eosinophils and that have not been associated previously with the eosinophil lineage (Fig 1).

Granule biogenesis

During their development, eosinophils synthesize large amounts of toxic granule proteins that must be posttranslationally modified and sequestered to maintain cell viability and ensure proper function. Three recent studies have highlighted novel points of regulation of granule biogenesis and its importance in eosinophil development and survival. The transcription factor X-box binding protein 1 (XBP1) is generally associated with highly secretory cells, such as plasma cells, Paneth cells, or pancreatic acinar cells, and plays a role in regulating the unfolded protein response by promoting transcription of genes encoding stress-response factors. XBP1 was not known previously to play a role in hematopoietic stem cells. However, after deleting *Xbp1* in the hematopoietic lineage, Bettigole et al[5] discovered that this transcription factor is uniquely essential for eosinophil development. The presence of the active and spliced form of *Xbp1* mRNA was found to peak during the GMP stage but remained prevalent until eosinophil maturation (Fig 1). Deletion of *Xbp1* in the hematopoietic lineage did not affect the proportion of GMPs but significantly reduced the proportion of EoPs and completely eliminated mature eosinophils. This effect appears to be due to defects in posttranslational maturation of the granule proteins major basic protein (MBP) and eosinophil peroxidase (EPX), disrupted granule formation, and downstream effects on GATA-1.

In addition to XBP1, the endogenous cysteine protease inhibitor cystatin F, also called leukocystatin, is necessary for proper granule biogenesis and eosinophil survival. Loss of cystatin F in mice uniquely affected the eosinophil compartment by leading to impaired granule formation and reduced cell viability (Fig 1).[6] The effect on eosinophils could be reversed by using pharmacologic inhibitors of cysteine proteases, suggesting that regulation of protease activity was necessary for proper maturation of granule proteins. MBP forms the electron-dense core of the secondary granules. Previously, it was not known how MBP was stored or mobilized in such a way to protect the eosinophil from its toxic effects. By using x-ray–free electron laser crystallography and granule core isolation, it was demonstrated that MBP is sequestered in a nondeleterious form as a nanocrystal and is mobilized during degranulation by means of acidification of the granule (Fig 1).[7]

Role of IL-33 in eosinophilopoiesis

The IL-1 family cytokine IL-33 signals through its receptor, ST2, which is expressed on a number of cell types involved in type 2 immunity, to initiate inflammatory responses. Although IL-33 has been shown previously to activate eosinophils, several recent studies examined the role played by IL-33 in promoting eosinophilopoiesis.

Anderson et al[8] examined how exposure of naive BALB/c mice to a common fungal aeroallergen, *Alternaria alternata*, not only causes eosinophil recruitment to the lung but also accelerates eosinophilopoiesis in the bone marrow. This phenomenon was ablated when the increased circulating IL-5 levels were neutralized or when the experiment was performed with ST2-deficient or type 2 innate lymphoid cell (ILC2)–deficient mice. Together, these data suggest that ILC2s in the context of fungal allergen exposure respond to IL-33 by secreting IL-5 that promotes eosinophilopoiesis (Fig 1). However, the importance of IL-33 in eosinophilopoiesis extends to a direct role under steady-state conditions.

Johnston et al[9] discovered that loss of IL-33 responsiveness in ST2-deficient mice reduced the rate of homeostatic eosinophilopoiesis. Conversely, IL-33 administration promoted eosinophil development both by increasing IL-5 production in the bone marrow and by expanding the number of IL-5 receptor α^+ eosinophil precursors (Fig 1).

REGULATION OF EOSINOPHIL LONGEVITY

Beyond the modulation of eosinophil production in bone marrow, eosinophil numbers can be regulated in the periphery in both cell-intrinsic and cell-extrinsic ways. Kotzin et al[10] showed that the long noncoding RNA *Morrbid* is present at high levels in mature eosinophils and other short-lived myeloid cells and that its deletion in mice leads to a loss of these cells through apoptosis in a cell-intrinsic manner (Fig 1). Furthermore, *Morrbid* levels were increased by prosurvival cytokine signaling in both mouse and human eosinophils from donors with hypereosinophilic syndrome (HES). In addition to their effects on *Morrbid* expression, another recent report found that prosurvival cytokines promoted eosinophil survival by upregulating Bcl-x_L in a manner prevented by an inhibitor of nuclear factor of κ light polypeptide gene enhancer in B cells (NF-κB) signaling.[11]

Accordingly, eosinophil-specific deletion of the endogenous inhibitor NF-κB inhibitor α (IκBα) resulted in constitutively active NF-κB and promoted Bcl-x_L expression and eosinophil survival. Bcl-x_L was both necessary and sufficient for eosinophil survival through overexpression studies. Thus prosurvival cytokines regulate eosinophil survival through several pathways that together modulate the balance of prosurvival and proapoptotic Bcl2 family members.

In a cell-extrinsic manner eosinophil longevity can be regulated through engagement of sialic acid–binding immunoglobulin-like lectin (Siglec) F in the mouse. Previous reports indicated the presence of endogenous lung Siglec-F ligands that were cytokine inducible and protease and sialidase sensitive. Kiwamoto et al[12] identified 2 glycoproteins from mouse lung, the mucins Muc4 and Muc5b, that display ligands for Siglec-F (Fig 1). They additionally showed that mucins isolated from mouse tracheal epithelial cells bind to unmasked Siglec-F on mouse eosinophils and induce their death. Finally, exacerbated eosinophilic airway inflammation was demonstrated in conditional Muc5b-deficient mice, indicating that this mucin contains Siglec-F sialoside ligands capable of resolving eosinophilic inflammation *in vivo*.

Another lectin with previously acknowledged immunomodulatory activities on other cell types, galectin-1, can also help resolve eosinophilic inflammation in the mouse.[13] Galectin-1 binds to LacNAc residues in O- or N-linked glycans on the cell surface. A recent study found that galectin-1 expression was induced in response to airway inflammation and bound to eosinophil cell-surface N-linked glycans. At low concentrations (≤0.25 μmol/L), galectin-1 promoted eosinophil adhesion to vascular cell adhesion molecule 1 and inhibited migration toward eotaxin-1. However, at higher concentrations (≥1 μmol/L), galectin-1 induced eosinophil apoptosis in a mitogen-activated protein kinase kinase–dependent and caspase-independent manner.

The prostaglandin D_2 receptor, chemokine receptor homologous molecule expressed on T_H2 lymphocytes (CRTH2), can be used as a marker of a number of type 2 immune cells, including eosinophils. Because asthma pathogenesis involves more than a single cell type or inflammatory mediator, Huang et al[14] focused on CRTH2 as a therapeutic target to deplete these cells. They developed a mouse expressing human CRTH2 on eosinophils, basophils, and ILC2s and an antibody with enhanced antibody-dependent cell cytotoxic activity against the protein. They showed that treatment of these mice with the antibody depletes human CRTH2-expressing cells and reduces type 2 immunity in response to *Nippostrongylus brasiliensis* helminth infection.

MOLECULAR PROFILING AND PHENOTYPIC DIVERSITY OF EOSINOPHILS

Molecular profiling can be used to compare eosinophil populations from different conditions or patients with one another in an unbiased manner. This more complete picture of molecules present in eosinophils can also be used to generate new hypotheses or help answer existing questions. Previously, relatively few of the proteins present in eosinophils had been annotated. Hence Wilkerson et al[15] sought to define the proteome of the resting peripheral blood eosinophil and analyze changes in response to IL-5 stimulation by phosphoproteomics. Their analyses expanded the number of proteins identified in the eosinophil to 7086 and ranked them according to estimated relative abundance, providing a wealth of information to mine for future studies. The phosphoproteomics data detected 220 phosphoisoforms that were significantly changed after 5 minutes of IL-5 stimulation, including some that were unanticipated and have not yet been characterized in eosinophils.

By flow sorting peripheral blood eosinophils from patients with asthma, patients with other eosinophilic disorders, or healthy subjects and performing RNA microarrays, Barnig et al[16] aimed to determine whether there were transcriptional differences that defined eosinophil populations in these patients. They found that the transcriptional profile of eosinophils from patients with asthma was similar to that from patients with peripheral eosinophilia related to dermatologic disease, parasitosis, or pulmonary aspergillosis. Eosinophils from patients with asthma were found to have higher mRNA levels for *IL2RA*, *IL10RA*, and *LIPA*, for example, and lower levels for *IL8*, *CCL3*, and *AQP9*, all of which were validated by using real-time RT-PCR.

Beyond the distinctions between eosinophil populations in different patients and disease states, there have been several recent studies highlighting the phenotypic diversity of eosinophils within an organism. Although lung-resident eosinophils (rEos) had been acknowledged before, no reports had fully characterized this population. A report by Mesnil et al[17] showed that this population in mice can be distinguished from recruited inflammatory eosinophils (iEos) on the basis of surface marker expression (rEos are Siglec-$F^{int}CD62L^{+}CD101^{low}$ cells and iEos are Siglec-$F^{high}CD62L^{-}CD101^{high}$ cells, Fig 1). An eosinophil population that resembled the lung-resident population could also be identified in the blood. Additional experiments supported a regulatory role for the rEos subset through suppressive effects on dendritic cell maturation and function. They found evidence of a similar lung-resident population in human subjects (Siglec-$8^{+}CD62L^{+}IL\text{-}3R^{low}$ rEos vs Siglec-$8^{+}CD62L^{low}IL\text{-}3R^{high}$ iEos), but the function of these eosinophils, compared with others, remains to be defined.

Another group examined mouse eosinophil subsets in the lung in response to allergen sensitization and challenge.[18] Based on this inflammatory context, these subsets are depicted in Fig 1 as probable subdivisions of the iEos subset, although this has not been demonstrated definitively. Although Gr-1 expression is typically associated with neutrophils, Percopo et al[18] found a distinct subset of eosinophils in the lung that coexpress this marker with Siglec-F, are absent in eosinophil-deficient ΔdblGATA mice, and produce a set of chemokines and cytokines that differs from their more numerous Gr-1^{-} counterparts.

Finally, Abdala Valencia et al[19] examined phenotypes of eosinophils recruited to distinct lung compartments (parenchyma vs airways) in response to allergen sensitization and successive challenges. They found an intriguing accumulation of a Siglec-$F^{high}CD11c^{low}$ eosinophil population (compared with an initial Siglec-$F^{int}CD11c^{-}$ phenotype) in the lung tissue and that only the CD11c-bearing eosinophils localized to the airway, suggesting a possible role for this integrin in migration to this specific lung compartment.

EOSINOPHILS AND MECHANISMS OF DISEASE PATHOGENESIS IN PRECLINICAL MODELS

Diseases of the skin and airways

Eosinophils can play a role in contactant-induced itch based on results from trimellitic anhydride treatment of wild-type (WT) or

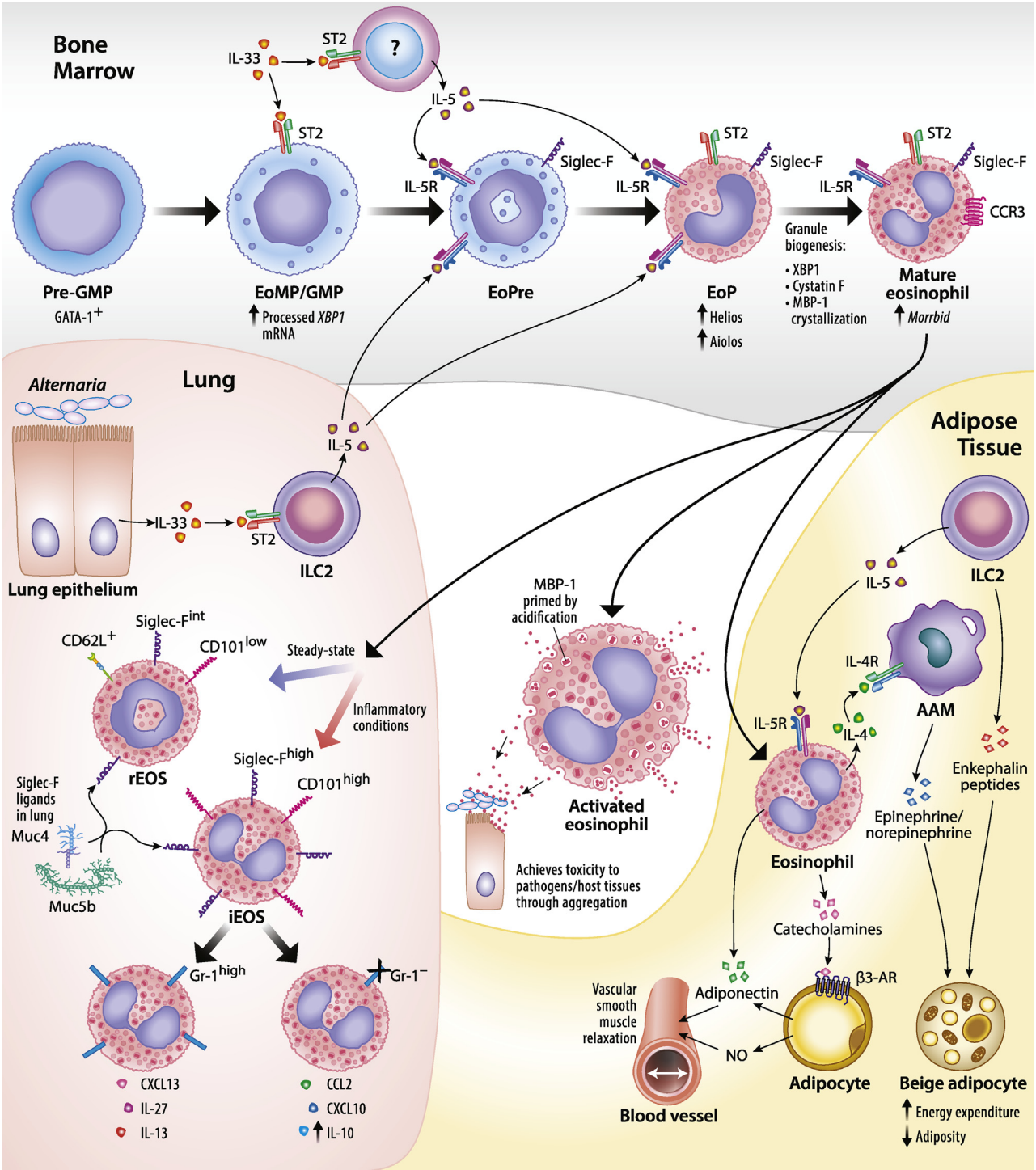

FIG 1. Recent advances in our understanding of eosinophil development, subset diversity, and function in peripheral tissues. *Bone Marrow*, Eosinophils develop from GATA-1$^+$ pre-GMPs in the bone marrow. These pre-GMPs give rise to GMPs that (at least in mice) respond to IL-33 through the ST2 receptor, which promotes eosinophil development and IL-5 receptor α *(IL-5Rα)* expression. GMPs express higher levels of processed *XBP1* mRNA, which is essential later in development. These GMPs give rise to Siglec-F$^+$IL-5Rα$^+$ mouse eosinophil precursors *(EoPres)*. Additionally, IL-33 promotes eosinophil development by inducing IL-5 expression from other bone marrow cells, acting on EoPres and EoPs, which then follow EoPres in lineage development. EoPs express higher levels of Helios and Aiolos, members of the Ikaros family of transcription factors, which can play a role in regulating gene expression during eosinophil development and remain highly expressed in mature mouse eosinophils. Proper granule maturation requires expression of the transcription factor XBP1, inhibition of cysteine protease activity by cystatin F, and crystallization of the granule protein MBP-1 in a nontoxic form. Improper granule maturation can lead to loss of cell viability and blockade of eosinophil development. The long noncoding RNA *Morrbid* is highly expressed in

eosinophil-deficient *PHIL* mice.[20] The authors of this study found that in the absence of eosinophils, inflammatory cell infiltration, tissue remodeling, increased skin innervation, and itching caused by trimellitic anhydride treatment were all reduced, indicating a role, either direct or indirect, of eosinophils in this response.

Although eosinophils are not normally present in healthy skin, they are abundant in patients with a variety of skin diseases. The mechanism of eosinophil recruitment to the skin in an IgE-dependent model of eosinophilic dermatitis was investigated by Cheng et al[21] in a recent study. Eosinophil skin infiltration in response to passive sensitization and cutaneous antigen exposure was unaffected in mast cell–deficient mice but was ablated in basophil-deficient mice. The role of basophils in this response was corroborated also in an active sensitization model and was shown to be dependent on basophil-derived IL-4. In response to IL-4, endothelial cells express vascular cell adhesion molecule 1, which is important for eosinophil entry.

It has been assumed that eosinophil degranulation plays a role in the airway hyperresponsiveness and tissue remodeling observed in asthmatic patients. However, Jacobsen et al[22] found little evidence for this using a mouse model of chronic T_H2 lung inflammation in which peripheral T cells constitutively express IL-5 and the central airway epithelium expresses human eotaxin-2 to drive eosinophil recruitment. They reported that in this model EPX is responsible in part for mucin induction in the airway, but neither EPX nor MBP plays a major role in inflammatory cell infiltration, collagen deposition, or methacholine-induced airway hyperresponsiveness. Instead, they showed that IL-13 is necessary for these eosinophil-dependent effects.

Diseases of the digestive tract

Although eosinophils home in large numbers to the gut during steady-state conditions, their role in promoting inflammatory conditions, such as chronic colitis, was not yet well defined. Griseri et al[23] showed that eosinophils, but not neutrophils, were required for maximal inflammation in a *Helicobacter hepaticus*– and IL-10 receptor blockade–dependent model of colitis. GM-CSF produced in response to the infection was responsible for the influx of iEos, which released IL-13, TNF-α, and EPX, the last of which promoted colitis. In a dextran sulfate sodium–induced mouse model of colitis, Moshkovits et al[24] demonstrated that CD300f, which was expressed on eosinophils rather than inflammatory monocytes, was partially responsible for disease severity. This receptor appeared to act in an activating or costimulatory fashion on eosinophils by promoting IL-6 and TNF-α production in response to stimulation with heat-killed *Escherichia coli*. In contrast to the previously discussed roles of eosinophils in promoting disease in the skin, airways, and digestive tract, Sugawara et al[25] showed that small intestinal eosinophils uniquely (compared with blood or bone marrow eosinophils) expressed high levels of IL-1 receptor antagonist in response to GM-CSF signaling and thereby suppressed inflammatory T_H17 responses in the intestine.

Paired immunoglobulin-like receptor B (PIR-B) is an inhibitory receptor expressed on the surfaces of eosinophils and other cells. A study by Ben Baruch-Morgenstern et al[26] showed that PIR-B suppresses eosinophil accumulation and activation in the esophagus in a mouse model of eosinophilic esophagitis (EoE) involving lung-specific and doxycycline-inducible IL-13 expression. The authors found that in PIR-B–deficient mice eosinophils were present in the esophagus in greater numbers and tissue remodeling was enhanced, exacerbating the deleterious effect of eosinophils in this model.

Roles of eosinophils in patients with cancer

In addition to inflammatory diseases, 2 new studies have shown diverse roles for eosinophils in tumor rejection and metastasis. The first, a study by Zaynagetdinov et al,[27] showed that loss of IL-5 impeded tumor metastasis to the lung in a number of transplantable tumor models. This phenomenon was reversed partially by administration of bone marrow–derived eosinophils back into IL-5–deficient mice. Tumor-induced CCL22 production by eosinophils in this model was necessary for the recruitment of regulatory T cells that established a suitable microenvironment for tumor metastasis, indicating that eosinophil activity can be exploited by tumors to promote metastasis.

In contrast, another study demonstrated that eosinophils were important in anticancer immunity.[28] Using a chicken egg ovalbumin–expressing B16 melanoma tumor model, the authors showed that regulatory T-cell depletion led to tumor rejection in an eosinophil-dependent manner and that adoptive transfer of OT-I chicken egg ovalbumin–reactive CD8 T cells with IFN-γ–and TNF-α–activated eosinophils was sufficient to cause tumor rejection, whereas neither cell population alone was sufficient. The effect of the eosinophils in this model was likely due to promotion of $CD8^+$ T-cell infiltration through production of

eosinophils and other short-lived myeloid cells and has been found to prevent cell death by inhibiting transcription of the proapoptotic Bcl2 family member *Bim*. *Activated eosinophil*, In the periphery eosinophil activation leads to granule acidification, thereby priming MBP-1 by altering its conformation. On release, MBP-1 exerts a toxic effect on pathogens and host tissues through aggregation. *Lung*, Distinct eosinophil subsets exist in the mouse lung distinguishable by surface marker expression: lung rEos that traffic to the lung under steady-state conditions and recruited iEos, which can be further subdivided by Gr-1 expression that corresponds to distinct sets of chemokine and cytokine products. rEos appear to possess regulatory properties, such as inhibiting maturation of type 2–biased allergen-loaded dendritic cells, that iEos do not. Sialosides on the mucins Muc4 and Muc5b bind to Siglec-F to induce eosinophil apoptosis in the mouse airway. ILC2s in the lung, in response to IL-33 signaling caused by *Alternaria* species exposure, produce IL-5 that promotes eosinophilopoiesis. *Adipose Tissue*, IL-5–activated mouse eosinophils indirectly promote energy expenditure in beige adipocytes by inducing release of epinephrine and norepinephrine from AAMs through IL-4 secretion. ILC2s produce IL-5 but also act directly and independently on beige adipocytes through release of enkephalin peptides. Both directly and indirectly, eosinophils cause blood vessel relaxation in PVAT through adiponectin and catecholamine release, respectively. The catecholamines signal through β_3-adrenergic receptors *(β3-AR)* on adipocytes to cause vessel relaxation through nitric oxide *(NO)* and adiponectin. Illustration were provided by Jacqueline Schaffer.

chemokines by eosinophils in the tumor microenvironment and restoration of normal vascular architecture through an undefined mechanism.

Regulatory roles of eosinophils in preclinical disease models

In contrast to their expected role in allergic airway inflammation, Takeda et al[29] showed that eosinophils are (1) not necessary for the development of airway hyperresponsiveness in mice in response to repeated allergen challenges and (2) instead necessary for the resolution of such inflammation. This group arrived at this surprising finding by using an allergic sensitization and challenge model with assays after either 7 or 11 challenges in both WT and eosinophil-deficient *PHIL* mice. *PHIL* mice exhibited not only the same degree of airway hyperresponsiveness after 7 challenges but also a trend toward greater hyperresponsiveness after 11 challenges and significantly more mucin deposition. By restoring either WT or IL-10–deficient eosinophils to *PHIL* mice, the authors found that eosinophils, through their production of IL-10, exert a beneficial immunoregulatory function that helps resolve airway inflammation.

Inflammatory arthritis occurs through type 1– and type 17–polarized immune responses in the context of autoimmunity. Thus the systemic introduction of stimuli that might skew immunity toward a type 2 response can assist with the resolution of such responses. A study by Chen et al[30] made use of *N brasiliensis* helminth infection and systemic IL-5 overexpression to determine whether this might occur in 2 mouse models of inflammatory arthritis. The authors found that these stimuli reduced disease severity in association with an influx of eosinophils into the affected joints. Amelioration of joint disease was dependent on IL-4 and IL-13 signaling, and use of eosinophil-deficient ΔdblGATA mice led to a partial reduction in this protective effect, implicating eosinophils directly in this protective effect.

ROLES OF EOSINOPHILS IN ADIPOSE TISSUE

Recently, diverse roles for eosinophils in the homeostatic regulation of adipose tissue (AT) have come to light. Eosinophils and alternatively activated macrophages (AAMs) in AT have been implicated in the induction of a brown adipocyte phenotype in white adipocytes, a process known as browning or beiging. These "beige" adipocytes increase energy expenditure and thermogenesis by expressing the uncoupling protein UCP1 and thereby reduce adiposity. A study by Brestoff et al[31] refined the relationships between the immune cells that regulate this process. The authors found that ILC2s, which are present in AT, regulate the recruitment and activity of eosinophils and AAMs through IL-33–induced production of IL-5 and IL-13. Eosinophils act further on AAMs by producing IL-4. Both AAMs and ILC2s produce signaling molecules that act directly on adipocytes to induce beiging, including AAM-derived epinephrine and norepinephrine and ILC2-derived enkephalin peptides (Fig 1).

A study by Suárez-Zamorano et al[32] dealing with the same topic found that microbiome depletion, either through antibiotic treatment or by using germ-free mice, leads similarly to fat browning. Through an unknown mechanism, microbiome depletion leads to local production of IL-4, IL-5, and IL-13 in the inguinal subcutaneous AT and recruitment and conversion of eosinophils and AAMs in an IL-4 receptor α–dependent manner. These data suggest that eosinophils promote leanness, at least in mice.

Beyond the role of eosinophils in regulating AT metabolism, eosinophils can play an important role in regulating the anticontractile function of perivascular adipose tissue (PVAT). A study by Withers et al[33] showed that constriction of small mesenteric arteries is reduced in the presence of PVAT in lean mice, whereas this effect is lost in obese mice, in which there are fewer PVAT eosinophils. In an *ex vivo* model of artery constriction, WT or inducible nitric oxide synthase–deficient eosinophils, but not those lacking adiponectin expression, induced vessel relaxation. Finally, the authors showed that eosinophils contain tyrosine hydroxylase and catecholamines and found that an inhibitor of tyrosine hydroxylase partially eliminates the relaxation effect induced by eosinophils. Together, these data indicate that eosinophils act directly on vascular smooth muscle through adiponectin expression and indirectly through adipocytes through catecholamine secretion to induce vessel relaxation (Fig 1).

Many studies of the relationship between eosinophils and AT have made use of models in which eosinophils are systemically absent or in excess. Although eosinophils are present in the AT of lean WT mice and are reduced in the AT of obese WT mice, Bolus et al[34] noted augmented eosinophil levels in the AT of both lean and obese CCR2-deficient mice, as well as the peritoneal cavity, but not in the blood, spleen, liver, or bone marrow, providing a promising system to study the local effects of increased eosinophil levels.

EOSINOPHILIA, HYPEREOSINOPHILIA, AND HESs

Eosinophilia is defined as an increase in blood eosinophil counts to greater than the upper limit of normal, typically greater than 0.5×10^9/L. The term hypereosinophilia is used to designate eosinophil counts of 1.5×10^9/L or greater, mainly because the differential diagnosis narrows. HESs are defined by persistent hypereosinophilia along with eosinophil-related organ involvement that is otherwise not attributable to any other diagnosis. The reader is directed to several excellent recent reviews focused on the diagnosis and treatment of patients with eosinophilia or hypereosinophilia.[35,36]

Important advances in the HES field included a retrospective analysis from the Mayo Clinic defining the risk of hematologic malignancies in patients with eosinophilia or hypereosinophilia, which fortunately was low over a 13-year period (0.2% of 2642 patients identified) and mainly consisted of T-cell malignancies in those with hypereosinophilia.[37]

Khoury et al[38] reported on 4 patients with an extremely rare cyclic form of HES called Gleich syndrome or episodic angioedema with eosinophilia. They identified several novel features associated with the unique, roughly monthly periodicity of their recurrences, namely evidence for T-cell clonality with the presence of an abnormal $CD3^-CD4^+$ T cell population that is reminiscent of what is often seen in the lymphocytic variant of HES, except that there was a concomitant cycling increase in numbers of cells other than eosinophils (eg, lymphocytes and neutrophils) along with cyclic production of IL-5 and other type 2 cytokines that preceded each episode. What remains baffling is why this unique periodic pattern occurs.

These same primary authors reported separate findings from a study treating patients with HES with imatinib regardless of

imatinib-responsive platelet-derived growth factor receptor α (PDGFRA)–associated mutation status. As expected, they saw 100% response rates in those with the diagnosis of FIP1L1-PDGFRA-myeloid neoplasm. Interestingly, they observed approximately 50% response rates in PDGFRA-negative HES with 4 or more criteria suggestive of a myeloid neoplasm, which strongly implicates the presence of other gain-of-function mutations responsive to tyrosine kinase inhibition. In marked contrast, none of the subjects with steroid-refractory, PDGFRA-negative HES with less than 4 criteria suggestive of a myeloid neoplasm responded to imatinib. These data suggest that the presence of 4 or more myeloid features, even in the absence of the classic FIP1L1-PDGFRA deletion mutation, was the best predictor of imatinib responsiveness, and thus these are the patients in whom a trial of imatinib might be warranted.[39]

Finally, although discussion in this particular section of the review might be premature, it was intriguing to read that the oral drug dexpramipexole (an enantiomer of pramipexole, a dopamine agonist approved for the treatment of Parkinson and restless leg syndrome), while being tested in clinical trials for efficacy in amyotrophic lateral sclerosis, did not have clinical efficacy but on safety monitoring was noted to cause a slow-onset, sustained, and selective eosinopenia.[40] As a result of this unexpected finding, dexpramipexole is undergoing clinical trials based on its antieosinophil properties and, if found to be safe and effective, could become the first oral agent to selectively reduce eosinophil numbers.

Two additional studies examined eosinophilic drug-induced reactions, one focusing on outpatient and the other on inpatient events. One was a prospective study of more than 800 former inpatients who continued to receive antibiotics in the outpatient setting and revealed an unexpectedly high rate of eosinophilia (25%) and a nearly 1% rate of drug reaction with eosinophilia and systemic symptoms syndrome.[41] Most episodes were attributable to the use of antibiotics, and those most commonly implicated were vancomycin, penicillin, rifampin, and linezolid. Development of eosinophilia was associated with an increased risk of rash and renal function abnormalities but not liver injury.

The other study, based at a single tertiary care hospital in Spain that followed patients prospectively during their hospitalization, found an incidence of eosinophil-associated drug reactions of 16.67 per 10,000 admissions and involved a wide range of drugs. Slightly more than half of the 274 patients in whom the eosinophil count was 0.7×10^9/L or greater were asymptomatic. However, 44% of patients had skin, soft tissue, and/or organ involvement, with about half of these patients having potential drug reactions with eosinophilia and systemic symptoms syndrome. The authors also concluded that the main predictors of severity were an earlier appearance and greater degree of eosinophilia.[42]

ROLE OF EOSINOPHILS IN AIRWAYS DISEASE

Upper airway

Among eosinophilic diseases of the upper airways, chronic rhinosinusitis (CRS), especially the subset accompanied by nasal polyposis (chronic rhinosinusitis with nasal polyposis [CRSwNP]), remains a particularly recalcitrant disorder. Although our understanding of its pathophysiology continues to improve,[43] our ability to monitor disease activity and provide effective and long-lasting treatment remains unsatisfactory. Several recent publications found useful correlations between eosinophil-related biomarkers assessed in the upper airways and either upper or lower airways eosinophilic inflammation. Analysis of microparticles originating from various cellular sources, which were obtained by sampling nasal secretions in patients with CRS, provided unique signatures that appear to distinguish between subsets of CRS. For example, increased numbers of microparticles from activated mast cells, basophils, and platelets were seen more commonly in those with CRS with aspirin-exacerbated respiratory disease than in those with CRS alone, whereas microparticle signatures consistent with epithelial injury were greater in patients with aspirin-exacerbated respiratory disease and patients with CRS without nasal polyps than in patients with CRSwNP.[44]

To examine local mucosal anti-inflammatory responses, Jia et al[45] explored the presence of glycan ligands for the proapoptotic receptor Siglec-8 on eosinophils in upper airway tissue samples from healthy subjects and patients with CRS and showed that specific, high-molecular-weight, sialic acid–containing ligands were abundant in submucosal glands and increased in patients with CRS. This could represent an endogenous pathway for controlling local eosinophilic inflammation.

Proton pump inhibitors (PPIs) are commonly used to treat gastroesophageal reflux disease and some forms of EoE, and therefore it was interesting to read that these agents at physiologically relevant concentrations also inhibit IL-13–induced epithelial expression of the important eosinophil chemoattractant CCL26 *in vitro* and do so through the nongastric H,K-ATPase pathway. Additionally, patients with CRS receiving PPIs were found to have lower CCL26 levels compared with those not taking PPIs. One possible conclusion for this study is that PPIs might be beneficial in reducing eosinophilic inflammation through a pathway that is independent of its effects on gastric acidity.[46]

Experiments sampling the upper airway as a proxy for the lower airway included one study in which levels of EPX in secretions obtained from the nasal passages and pharynx of patients with inadequately controlled asthma correlated with sputum eosinophil counts.[47]

Finally, in the realm of novel therapeutic approaches, particularly promising was a study of the efficacy of dupilumab, an antibody to the IL-4 receptor α subunit that blocks the activity of both IL-4 and IL-13, in patients with CRSwNP inadequately controlled with intranasal steroids.[48] Compared with the nasal steroid–only treatment group, significant improvements were seen in polyp burden scores and sense of smell in the nasal steroid plus dupilumab–treated group. Although longer and larger studies are needed, these data clearly demonstrate the importance of the IL-4/IL-13 axis in patients with CRSwNP.

Lower airway

Monitoring or predicting disease development, activity, and response to therapy. Sverrild et al[49] reported fascinating correlative results related to the diversity of the airway microbiome in patients with eosinophil-low and eosinophil-high asthma based on the 16S rDNA sequences detected in BAL fluid from each patient. The study found that microbial diversity was significantly lower in patients with eosinophil-low asthma and that numerous statistically significant differences existed with respect to the abundance of several microbial genera. No such differences were found with respect to patients with neutrophil-high

TABLE I. Newly approved drugs and others in development that target eosinophils either directly or indirectly that are mentioned in this article

Drug	Form	Drug target	Approved indication (or recent clinical trial)
Mepolizumab	Humanized IgG_1 administered subcutaneously	IL-5	Asthma age ≥12 y (and others)
Reslizumab	Humanized IgG_4 administered intravenously	IL-5	Asthma age ≥18 (and others)
Dupilumab	Human IgG_4 administered subcutaneously	IL-4 receptor α, blocking both IL-4 and IL-13	Adults with atopic dermatitis (and asthma and CRSwNP)
Benralizumab	Humanized afucosylated IgG_1 administered subcutaneously	IL-5 receptor α	Asthma and others
Dexpramipexole	Oral small molecule	Unknown	CRSwNP and others
Lebrikizumab	Humanized IgG_4 administered intravenously	IL-13	Asthma and others
Dectrekumab (QAX576)	Humanized IgG_1 administered intravenously	IL-13	Asthma, EoE, and others
SB010	Inhaled small molecule	GATA-3–specific DNAzyme	Asthma
Fevipiprant	Oral small molecule	CRTH2 (DP_2, type 2 prostaglandin D_2 receptor)	Asthma
Budesonide	Oral suspension; small molecule	Glucocorticoid receptor	EoE

or neutrophil-low asthma. Neither the underlying reason for such differences nor their relationship to disease progression is clear.

Wang et al[50] examined the influence of sputum mast cell subtypes on eosinophilia and asthma control in patients. They found that levels of the $MC_{T/CPA3}$ subtype, expressing both tryptase and carboxypeptidase 3, correlated with sputum eosinophil levels, increased fraction of exhaled nitric oxide levels, and poorer asthma control. The role of this mast cell subtype in establishment of eosinophilic asthma has not yet been defined.

Two IL-5–targeting therapies are now available for the treatment of eosinophilic asthma, namely mepolizumab and reslizumab (Table I). The primary end point for approval of both agents was a reduction in asthma exacerbations. Although we still do not know why neutralizing IL-5 (and, by inference, decreasing eosinophil numbers) reduces asthma exacerbations, and although these 2 biologics have never been compared directly in the same clinical trial, what is emerging in the literature is that when used in the right population of asthmatic patients, they are indeed effective. Such studies also provide a better sense of how eosinophil counts in the blood relate to eosinophil counts in the airways in asthmatic patients and how this information can influence treatment choice and outcomes.[51] Several reports, including some that were retrospective, showed that blood eosinophil counts predict treatment outcomes with mepolizumab use, especially in patients with severe asthma, including the observation that with a baseline blood eosinophil count of 0.15×10^9/L or greater, clinically meaningful reductions in exacerbation rates are achieved.[52,53] Additional findings included the observation that increased blood eosinophil counts of 0.45×10^9/L or greater, a higher cutoff than those used by the aforementioned clinical trials, predict sputum eosinophilia[54] and that levels of IL-13 and IL-5 in the serum are useful in identifying the eosinophilic asthma subset.[55]

Revisiting the concept of using sputum eosinophils to assess asthma control, Demarche et al[56] followed sputum eosinophilia longitudinally in a clinical practice setting and reported that in the subgroup of patients with intermittent or persistently eosinophilic asthma, a decrease of 3.4-fold or 4.3% in sputum eosinophil counts predicted improvement in asthma control, whereas an increase of 3.5% or 1.8-fold was associated with a deterioration of control. A prospective study done at Kaiser Permanente showed that in patients with severe uncontrolled asthma aged 12 years or greater, a blood eosinophil count of 0.4×10^9/L or greater was associated with a 1.55-fold risk of having 2 or more asthma exacerbations or asthma-related emergency department visits or hospitalizations over a 1-year period.[57]

Two studies examined blood eosinophil counts in patients with chronic obstructive pulmonary disease (COPD). One study from Copenhagen found that in patients in the general population with COPD followed for a median of 3.3 years, those with a blood eosinophil count of 0.34×10^9/L or greater had a 1.76-fold greater risk of severe exacerbations,[58] whereas a separate multicenter study found no influence of blood eosinophil counts on COPD exacerbation rates among those receiving either indacaterol plus glycopyronium or fluticasone plus salmeterol.[59]

Finally, several studies evaluated blood eosinophil counts in other unique circumstances. Anderson et al[60] reported that in children evidence of aeroallergen sensitization and a blood eosinophil count of 0.3×10^9/L or greater at the age of 2 years were associated with a 3.1- to 3.3-fold increased risk of having asthma at age 6 years, but blood levels of periostin were not predictive, perhaps because of bone-derived sources related to bone turnover in this younger age group. In exacerbation-prone asthmatic patients followed in the National Heart, Lung, and Blood Institute's Severe Asthma Research Program 3 study, blood eosinophil counts were positively associated with higher exacerbation frequency (1.6-fold for every log of eosinophil levels), and reminiscent of comments above, the presence of CRS was independently associated with a 1.7-fold increase in asthma exacerbation frequency.[61] One additional, retrospective, single-center report of 15 patients treated with bronchial thermoplasty for severe asthma found a 50% decrease in blood eosinophil counts (from a pretreatment mean of 0.33×10^9/L to a posttreatment mean of 0.17×10^9/L). This was associated with a significant reduction in median

numbers of emergency department and outpatient doctor's office visits but without any accompanying improvement in lung function.[62] Taken together, these studies consistently report associations between eosinophil counts and CRS, asthma, and asthma control, especially in certain cohorts of asthmatic patients, such as those at the severe end of the spectrum.

Asthma studies involving eosinophil-related treatments that are not yet approved. The first study to be mentioned in this section is a successful trial of mepolizumab in patients with eosinophilic granulomatosis with polyangiitis (EGPA; formerly known as Churg-Strauss syndrome).[63] It is mentioned here because the study involved the subcutaneous administration of 300 mg every 4 weeks instead of the currently approved and available dose of 100 mg every 4 weeks. This multicenter controlled trial enrolled 136 subjects with stable disease receiving a stable prednisolone or prednisone dose along with standard EGPA care. Treatment with mepolizumab led to a greater likelihood of having 24 to 48 weeks of disease remission (approximately 30% receiving active drug vs approximately 3% receiving placebo), and 44% of the participants in the mepolizumab group compared with 7% of those in the placebo group ended up needing an average daily dose of prednisolone or prednisone of 4 mg/d or less during the last 4 weeks of this year-long study. Somewhat disappointing was the finding that remission did not occur in 47% of the participants in the treated group, although this was clearly better than rates of 81% for those receiving placebo.

In addition to IL-5–targeting antibodies, other agents that directly or indirectly target eosinophils are in various stages of clinical development (Table I). Particularly advanced are trials with benralizumab (anti–IL-5 receptor antibody), dupilumab, and lebrikizumab (anti–IL-13 antibody). Various multicenter, international, phase 3 studies in patients with severe and poorly controlled asthma with benralizumab consistently show marked and sustained reductions in eosinophil (and basophil) counts, as well as safety and efficacy. Favorable outcomes included improvements in asthma control, reductions in asthma exacerbations and oral steroid–sparing effects, and some inconsistent evidence of improvement in lung function.[64-66] When studied in patients with mild-to-moderate persistent asthma receiving inhaled corticosteroids, a small but statistically significant improvement of 80 mL in prebronchodilator FEV_1 was seen at 12 weeks in the benralizumab-treated group but not in the placebo group.[67] Whether this is clinically significant and has an effect on disease activity or progression is unknown, but the modest degree of improvement does not yet justify its use in this population with milder asthma.

As a follow-up to a successful initial trial of dupilumab in patients with persistent moderate-to-severe asthma and a blood eosinophil count of 0.3×10^9/L or greater or sputum eosinophil counts of 3% or greater,[68] a subsequent larger phase 2b study was completed examining the benefits of adding dupilumab to medium- to high-dose inhaled corticosteroids plus a long-acting β_2-agonist in patients with uncontrolled persistent asthma. For this study, subjects were enrolled regardless of their eosinophil counts. Particularly striking was the finding that, on average, dupilumab improved FEV_1 by 0.39 L and reduced severe exacerbations by 70%; this occurred irrespective of baseline eosinophil counts.[69] The results with dupilumab are in contrast to disappointing results from 2 international phase 3 studies, each with more than 1000 subjects with uncontrolled asthma despite using inhaled corticosteroids and at least 1 controller medication, who were randomized to receive placebo or lebrikizumab. This study did not meet its primary end point, which was a reduction in exacerbations over a 1-year period in patients with high blood eosinophil counts or periostin levels. This was due to the fact that one study reached statistical significance while the other one did not.[70]

Given the contrast between these dupilumab and lebrikizumab results, one is tempted to conclude that targeting both IL-4 and IL-13 is better than targeting IL-13 alone and/or that targeting the receptor rather than the cytokine is more efficacious.

Two other agents in clinical trials that are not biologics and target very distinct pathways deserve mention based on recent publications. One is an inhaled agent that is an antisense DNA called SB010 designed to eliminate the master T_H2 transcription factor GATA-3. GATA-3 is expressed not only in T_H2 cells but also in mast cells, eosinophils, and other cells.[71] When inhaled once daily for 4 weeks in patients with mild allergic asthma with sputum eosinophilia before inhaled allergen provocation, Krug et al[72] showed that it reduced both early- and late-phase asthmatic responses. Further analyses in a subsequent report from the same group showed that protection from lung function decrease in this model was more marked, with increasing blood eosinophil counts and levels of exhaled nitric oxide.[73] The exact reason for the beneficial effects seen with SB010 on the acute and late response is unknown, but it did reduce serum IL-5 levels by a modest degree. Thus far, there are no published data from asthma clinical trials with SB010.

Separately reported were the results of a phase 2 single-center trial in patients with moderate-to-severe persistent asthma and sputum eosinophil counts of 2% or greater of twice-daily oral administration of fevipiprant (QAW039).[74] This drug is a small-molecule antagonist of the prostaglandin D_2 receptor, which is also known as CRTH2 or DP_2 and expressed on many cell types associated with type 2 inflammation, including eosinophils. After 12 weeks of treatment, which was well tolerated, there was a significant reduction in sputum eosinophil counts from a geometric mean of 5.4% to 1.1% in the fevipiprant group compared with a decrease from 4.6% to 3.9% in the placebo-treated group. Whether this agent is beneficial in asthmatic patients remains to be seen.

EOSINOPHILIC GASTROINTESTINAL DISORDERS: DIAGNOSIS, PATHOPHYSIOLOGY, AND TREATMENT

The term eosinophilic gastrointestinal disorder (EGID) refers to any disorder involving accumulation of an abnormal number of eosinophils within a particular region of the gastrointestinal tract and includes EoE, eosinophilic gastritis, eosinophilic colitis, and combinations, such as eosinophilic gastroenteritis. The prevalence of these disorders and the role played by food in causing these disorders decreases the further down one goes within the gastrointestinal tract, and therefore most of the attention has been paid to EoE. Endoscopy with biopsy remains the mainstay of diagnosis and assessment of disease activity. EoE is also diagnosed partly on the basis of a failed PPI trial, which differentiates it from PPI-responsive esophageal eosinophilia (PPI-REE), a disorder with unknown cause that otherwise shares many clinical characteristics with EoE.

Wen et al[75] used transcriptomics to compare the inflammatory conditions in the esophagus underlying these 2 disorders, as well as PPI-REE before and after PPI treatment. The study found that

patients with PPI-REE had transcriptomic profiles that were very similar to those of patients with EoE based on an analysis using an EoE diagnostic panel of 94 esophageal transcripts. However, these PPI-REE transcriptomic profiles returned almost entirely to a state similar to that of healthy control subjects after PPI treatment. Together, these data indicate that PPI-REE mimicry of the transcriptomic profile of EoE—as well as the eosinophilia and clinical features—is PPI-responsive.

Although IL-5 and IL-13 are thought to drive eosinophilia and tissue remodeling in patients with EoE, the cellular source of these cytokines had not been established. Doherty et al[76] examined the possibility that ILC2s in the esophagus can be expanded in the context of EoE and might therefore play a role in initiating these changes. They defined ILC2s in esophageal biopsy specimens on the basis of their surface molecule expression ($CD45^+Lin^-CRTH2^+$) and found that ILC2s were overrepresented in patients with active EoE and that ILC2 levels strongly correlated with eosinophil levels, implicating them in eosinophil recruitment.

An international study published by Safroneeva et al[77] confirmed that in adults with EoE, monitoring symptoms as an assessment of disease remission, compared with use of endoscopic and histologic findings, was only accurate about 60% to 65% of the time, underscoring the continued need for tissue monitoring to most accurately assess disease activity and remission.[77] However, in children the story might be different. Using a Pediatric Eosinophilic Esophagitis Symptom Score (version 2.0) reported by parents of children with EoE, it was determined that this clinical measurement tool was effective at capturing important symptoms and that the Pediatric Eosinophilic Esophagitis Symptom Score was useful and valid because values correlated well with histologic and other tissue-derived parameters of disease activity, especially among patients with dysphagia.[78]

Nevertheless, one important goal in the management of such patients with EoE, regardless of whether they are children or adults, would be advances in less invasive measures to monitor disease activity. One approach that avoids biopsy but still requires endoscopic sampling involved performing esophageal mucosal brushings to collect evidence of local eosinophil accumulation by measuring levels of EPX in these samples. Indeed, a very strong correlation was seen between these levels and peak numbers of eosinophils in traditional biopsy specimens.[79] One additional potential advantage of this approach, as pointed out by the authors, is the ability of the brushing technique to sample a larger and more comprehensive surface area of the esophageal lumen.

Taking a very different approach, Lingblom et al[80] sampled blood and performed extensive eosinophil flow cytometric phenotyping and mRNA analyses to look for any specific patterns that distinguished eosinophils in children and adults with or without EoE. Differences in surface levels of CD44, CD54, and/or CRTH2 on eosinophils and intracellular levels of mRNA for galectin-10 (formerly called Charcot-Leyden crystal protein) and the forkhead box protein 3 transcription factor proved useful in distinguishing between the various groups, although some differences were age related rather than disease related. Whether this type of analysis will be useful in diagnosis of patients with EoE (compared with other eosinophil-associated disorders) or assessment of disease activity will require further testing. Regardless, the authors postulate that different eosinophil phenotypes in adults versus pediatric patients with EoE might be the result of differing disease mechanisms or pathophysiology.

In another study a large cohort of children undergoing oral immunotherapy for food allergy was followed to determine whether those who had abdominal pain or vomiting (about 8% of all subjects) also had blood eosinophilia, a finding that might suggest development of EGID. They found an increased likelihood of vomiting, abdominal pain, or both in patients with a higher maximum blood eosinophil count.[81] By using receiver operating characteristic analysis, a blood eosinophil count of 1.14×10^9/L yielded 85% sensitivity and 73% specificity of having these gastrointestinal side effects. Because very few subjects underwent endoscopic evaluation, the risk of actually having an EGID could not be determined, but it was of interest to note that when the food dosage was lessened or stopped, gastrointestinal symptoms subsided and total eosinophil counts decreased by greater than 50%. The authors call these oral immunotherapy–induced gastrointestinal and eosinophilic responses and highlight the possibility that oral immunotherapy has the potential, at least in some patients, to cause inadvertent EGID-like disease.

Regarding recent advances in the treatment of EGID, the primary focus has been on EoE. One particularly important advance came in the form of perfecting food elimination diets, in which Kagawalla et al[82] found that in children with EoE, a 4-food elimination diet (cow's milk, wheat, egg, and soy) resulted in a 60% remission rate. When compared with prior publications, including some by this same group, this approach was nearly as effective as the previous commonly used 6-food elimination diet that also avoided peanuts, tree nuts, fish, and shellfish, and therefore one conclusion is that these latter foods are rare triggers of EoE in children.

Many of the other recent advances came in the form of studies looking at the effectiveness of swallowed corticosteroids, such as an oral budesonide suspension. These studies were separate multicenter trials, one in children and the other in both children and adults, as registered clinical trials to test the safety and efficacy of a new oral budesonide formulation being developed as a new agent that might one day receive US Food and Drug Administration approval for the specific indication of treating EoE. Both studies showed this treatment to be safe. The pediatric study found drug- and dose-related improvements in histologic findings but no difference in symptoms for those receiving drug versus placebo because all groups, including those receiving placebo, showed unexpected improvement.[83] In contrast, studies in both children and adults showed significant drug-related improvements in histology and symptoms.[84]

One additional study examined the effectiveness of the anti–IL-13 antibody dectrekumab (QAX576) in patients with EoE.[85] This was a 12-week trial involving monthly intravenous infusions, and the drug was well tolerated. Although the primary end point, which was based on the percentage of subjects with a greater than 75% decrease in endoscopic biopsy eosinophil counts, was not met, average eosinophil counts decreased by 60% with drug administration compared with a 23% increase in the placebo group, a statistically significant difference. Particularly striking was the observation that improvements in tissue eosinophil counts, mast cell counts, EoE-relevant transcript signatures, and markers of barrier function persisted up to 6 months, which was months after the drug was stopped. Although this antibody is no longer in development, these results seem to underscore an important role for IL-13 in EoE pathogenesis.

EOSINOPHILS AND SKIN DISEASE

Although developments in recent years in eosinophils and dermatology were limited in scope, several important articles appeared. One examined the pathophysiology of bullous pemphigoid (BP), an autoimmune skin disease characterized by subepidermal blister formation, autoantibodies to hemidesmosomal antigens, and blister eosinophilia with evidence of local eosinophil degranulation. Using an *ex vivo* human model, de Graauw et al[86] found that incubation of normal human skin sections with IL-5–activated eosinophils and BP autoantibodies was sufficient to induce dermal-epidermal junction separation pathognomonic of this disease. Whether targeting eosinophils in patients with BP will be efficacious is an intriguing question that remains to be determined.

Finally, a series of 3 landmark studies explored the safety and efficacy of dupilumab in adults with moderate-to-severe atopic dermatitis.[87-89] Its benefit in these trials was substantiated in a subsequent meta-analysis,[90] and dupilumab received US Food and Drug Administration approval in 2016 for this indication.

ADVANCES IN OUR UNDERSTANDING OF EOSINOPHILS AND EOSINOPHIL-ASSOCIATED DISEASES

Examples of recent advances in our understanding of basic eosinophil biology involve the following:

- the role of IL-33 in eosinophilopoiesis;
- the existence of multiple eosinophil subsets in the lung;
- mechanisms of granule protein processing;
- certain intrinsic and extrinsic pathways regulating eosinophil longevity; and
- homeostatic and regulatory roles played by eosinophils in AT and lung tissue.

Recent clinical advances regarding eosinophils and eosinophil-associated diseases include the following:

- findings regarding the association between increased eosinophil counts and asthma and COPD exacerbation risk;
- the efficacy of biologicals targeting IL-5 in patients with EGPA, IL-5 or IL-5 receptor α in certain populations of asthmatic patients, and IL-4 receptor α in patients with CRSwNP and atopic dermatitis; and
- intriguing and promising data regarding the use of PPIs, dexpramipexole, the antisense DNA agent SB010, the CRTH2 antagonist fevipiprant, and others.

WHAT REMAINS TO BE DETERMINED IN EOSINOPHILS AND EOSINOPHIL-ASSOCIATED DISEASES?

Key issues in basic eosinophil biology that remain incompletely defined/require further study include the following:

- specific functions of the distinct eosinophil subsets;
- relevance of eosinophils located within the AT as opposed to elsewhere in the body;
- whether these findings extend from mice to human subjects;
- mechanisms underlying some of the immunoregulatory activities of eosinophils;
- complete pathway of eosinophil hematopoietic development; and
- how eosinophil identity and activity is transcriptionally regulated in the periphery.

Key issues related to eosinophil-associated diseases that remain incompletely defined/require further study include the following:

- the mechanism underlying the periodicity of Gleich syndrome;
- identities of the other apparent gain-of-function mutations in patients with imatinib-sensitive PDGFRα mutation-negative HES;
- mechanisms by which targeting eosinophils reduces asthma exacerbations;
- which eosinophil-reducing or eosinophil-depleting agent works best and most safely in patients with which eosinophil-associated disease;
- whether these agents favorably or adversely affect important homeostatic and regulatory functions associated with eosinophils;
- development of therapies to treat CRSwNP more efficaciously and durably; and
- invention of less invasive diagnostic tests for EGID.

We thank Jacqueline Schaffer for her contribution of Fig 1. This review is dedicated to Dr James "Jamie" J. Lee, whose untimely death in 2017 caught the entire eosinophil community by surprise. Many of the experiments cited in this review could not have been accomplished without the generous collaborative contributions of his intellect and the unique reagents and animals developed in his laboratory.

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