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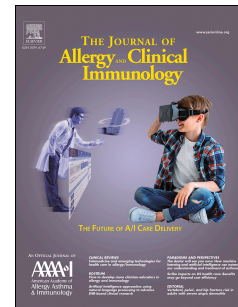
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2021 Year in Review: Spotlight on Eosinophils

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ABSTRACT

This review highlights recent advances in the understanding of eosinophils and eosinophilic diseases, particularly eosinophilic gastrointestinal diseases during the last year. The increasing incidence of diseases marked by eosinophilia has been documented and highlighted the need to understand eosinophil biology and eosinophilic contributions to disease. Significant insight into the nature of eosinophilic diseases has been achieved using next-generation sequencing technologies, proteomic analysis, and machine learning to analyze tissue biopsies. These technologies have elucidated mechanistic underpinnings of eosinophilic inflammation, delineated patient endotypes, and identified patient responses to therapeutic intervention. Importantly, recent clinical studies using monoclonal antibodies that interfere with type 2 cytokine signaling or deplete eosinophils point to multiple and complex roles of eosinophils in tissues. Several studies identified distinct activation features of eosinophils in different tissues and disease states. The confluence of these studies supports a new paradigm of tissue-resident eosinophils that have pro- and anti-inflammatory immunomodulatory roles in allergic disease. Improved understanding of unique eosinophil activation states is now poised to identify novel therapeutic targets for eosinophilic diseases.

36 **ABBREVIATIONS**

37	BZH	Basal zone hyperplasia
38	CD	Cluster of differentiation
39	ChIP	Chromatin immunoprecipitation
40	CLC	Charcot-Leyden crystals
41	CRSSNP	Chronic rhinosinusitis without nasal polyps
42	CRSsNP	Chronic rhinosinusitis with nasal polyps
43	EGID	Eosinophilic gastrointestinal diseases
44	EoC	Eosinophilic colitis
45	EoC	Eosinophilic duodenitis
46	EoE	Eosinophilic esophagitis
47	FDA	Food and drug administration
48	GWAS	Genome wide association studies
49	HES	Hypereosinophilic syndrome
50	IgE	Immunoglobulin E
51	IL	Interleukin
52	ILC	Innate lymphoid cell type
53	MCTR	Maresin conjugates in tissue regeneration
54	miR-1	microRNA 1
55	PPI	Proton pump inhibitor
56	ROC	Receiver operating characteristic
57	RNAseq	Ribonucleic acid sequencing
58	scRNAseq	Single cell ribonucleic acid sequencing
59	STING	Stimulator of interferon genes
60	TSLP	Thymic stromal lymphopoietin

Eosinophilic inflammation is a key feature of several inflammatory and allergic pathologies, and a new class of eosinophil-depleting therapies are having a dramatic effect on a variety of diseases (1-7). The full potential of these drugs is an important area of medical research, as there is still much unknown about the specific role of eosinophils in allergic and inflammatory diseases. Herein we discuss advances in eosinophil biology including eosinophilic gastrointestinal diseases; eosinophils in COVID-19; therapeutic interventions targeting eosinophils; features of tissue-specific eosinophils; interactions between eosinophils and other cell types; and current methodologies.

EOSINOPHILIC GASTROINTESTINAL DISEASES

Eosinophilic gastrointestinal diseases (EGID) including eosinophilic esophagitis (EoE), eosinophilic gastritis (EoG), and eosinophilic colitis (EoC) are rare pathologies for which research progress and clinical outcomes have benefitted from large, multi-site approaches and consortia that expand the patient pool, support clinical trials, and refine diagnostic criteria and standards of care (8, 9). There is significant interest in developing improved EGID diagnostic criteria that would be less invasive than sedation-requiring endoscopically derived tissue biopsies and that may more accurately predict treatment responses (10-13). Advances in unsedated transnasal esophagoscopy offer opportunities to less invasively diagnose and monitor EoE, especially at early timepoints after therapy (14). The diagnosis is confounded by the normal residence of eosinophils in the gastrointestinal tract during homeostasis (15).

Studies seeking to stratify patients with EGID have utilized tissue gene expression, serum biomarkers, and clinical parameters to identify molecular endotypes of EoE and EoG (8, 10, 16-18).

There has been exciting progress toward the understanding of eosinophilic diseases of the upper and lower gastrointestinal tract (Table I). Eosinophils are present in active EoE tissue

biopsies marked by normal-like, inflammatory, and fibrostenotic gene expression profiles, illustrating a role of eosinophils in multiple phases of disease (Figure 1) (16). Eosinophils are constitutively present in several tissues in the lower gastrointestinal tract, presenting a unique opportunity to understand the roles of eosinophils in homeostatic tissue maintenance as well as pathology. Recently, single-cell sequencing was obtained for eosinophils from esophageal and duodenal biopsies from patients with EoE during active disease and in remission (19). The authors identified differential eosinophil transcription factor utilization based on tissue localization and disease state, which supports distinct functions of eosinophils in different environments (19).

Eosinophilic Esophagitis

EoE is a recently recognized disease that is growing in prevalence (20, 21). Epithelial barrier breakdown leading to food and aero-allergen exposure is a key feature of EoE pathogenesis; this breakdown is largely driven by the type 2 cytokine interleukin 13 (IL-13), which exerts many functions, including inhibition of epithelial cell differentiation as well as loss of anti-proteases such as serine peptidase inhibitor kazal type 7 (SPINK7) in the esophagus (22-24). IL-13 signaling in the esophageal epithelium may be directly responsible for other epithelial pathologies observed in EoE, such as fibrosis and the hyper-proliferative state of epithelial cells that is associated with basal zone hyperplasia (BZH) (25, 26). It has been demonstrated that the calcium transporter ANO1 is increased in patients with EoE and positively correlated with eosinophil recruitment and BZH, and both murine and in vitro models illustrated that its upregulation in the epithelium is responsive to IL-13 (25).

Much attention has been given to the familial inheritance, diagnosis, and stratification of EoE (10, 16, 27-29). Expression of type 2 inflammatory genes, including *IL13*, is heterogeneous in biopsies from patients with active EoE (30). Despite differences in type 2 cytokines and eotaxin-

3 (*CCL26*) expression in patients with active EoE, eosinophilic inflammation is observed in normal-like, inflammatory, and fibrotic tissue gene expression profiles (16). This highlights a potential under-studied role of eosinophils in multiple disease states.

EoE was originally defined by non-responsiveness to proton pump inhibitors (PPI), but new diagnostic guidelines have removed this caveat (31). This change is supported in part by evidence that PPIs, in addition to decreasing gastric acid, also inhibit eotaxin production and other pro-inflammatory responses of the esophageal epithelium in the presence of IL-13 (11, 32, 33). Additional studies have demonstrated direct effects of PPIs on granulocytes. For instance, in both human and murine mast cells, omeprazole was associated with impaired mast cell maturation, degranulation, and cytokine secretion (34).

Eosinophilic Gastritis

As in other inflammatory and allergic diseases, the incidence of EoG is increasing over time, and there is substantial interest in the diagnosis and progression of EoG. It has recently been shown that in EoG, as in gastroenteritis and eosinophilic colitis, eosinophilic inflammation is observed in tissues outside the primary disease site, pointing to unknown shared inflammatory mechanisms (35). Eosinophil-depleting therapy targeting Siglec-8 has recently demonstrated efficacy in decreasing histological eosinophil count as well as symptoms of EoG (36).

Genetic and pathologic readouts of EoG have been recently reported in a large, multi-site cohort. RNA sequencing (RNAseq) analysis of whole tissue biopsies yielded hundreds of differentially expressed genes in EoG compared with control samples, and a subset of these genes were validated to develop a 48-gene panel that sensitively distinguished EoG from non-EoG individuals (10). Additionally, serum biomarkers were tested for their ability to sensitively detect EoG, although receiver operating characteristic (ROC) curves continue to support greater

139 sensitivity with tissue transcriptional analysis (10). It is interesting to note that two of the three
140 serum biomarkers that contributed to the serum EoG score were eotaxin-3 and IL-5.

COVID-19

There has been significant interest in eosinophils in the context of infection course and vaccination strategy for SARS-CoV2. Eosinophils may be effector cells in adverse reactions to vaccination, as had been observed in previous vaccination strategies for SARS-CoV-1 (37, 38). As in other viral diseases, eosinophils may participate in a hyper-inflammatory state and 'cytokine storm' that are associated with severe disease (39). However, data published early in the pandemic pointed to eosinopenia in patients hospitalized with SARS-CoV-2 (37). Restoration of eosinophils and other leukocytes was predictive of patient recovery, whereas progressive neutrophilia was associated with poor outcomes (40). A recent retrospective study reported that patients with elevated circulating eosinophils were less likely to be hospitalized with COVID-19, and eosinophilia during hospitalization was associated with decreased mortality (41). On the other hand, severe cases were reported that included profound eosinophil recruitment to lung vasculature, parenchyma, and airways, which was not positively correlated with a robust type 2 inflammatory response and which responded to systemic steroid administration (42).

Asthma has typically been a risk factor for pulmonary infectious disease; however, early data regarding the severity of COVID-19 in patients with asthma was conflicting (37, 43, 44). Non-eosinophilic asthma, which is associated with low expression of type 2 immune genes and low blood eosinophil counts, was associated with elevated expression of the viral receptor ACE2 and therefore elevated risk of severe disease (43). Conversely, in type 2-mediated diseases, including allergic rhinitis, eosinophilic asthma, and EGID, IL-13-driven downregulation of ACE2 has been observed and may account, in part, for the somewhat protective effect of these comorbidities in SARS-CoV2 infection and COVID-19 outcomes (45-47).

CLINICAL INTERVENTIONS TARGETING EOSINOPHILS

Therapeutic interventions targeting several components of inflammatory processes in eosinophilic diseases have received widespread attention in recent years (1, 48, 49). Monoclonal antibodies have been approved to target components of the type 2 cytokine milieu (e.g., IL-4 receptor α [R α], IL-5, IL-13, immunoglobulin E [IgE]) or to target and deplete eosinophils through antibody-dependent cytotoxicity (IL-5R, Siglec-8) (50, 51). Full implementation of therapeutic targeting of eosinophils will benefit both clinical insight into patient responses and improved understanding of basic eosinophil functions in health and disease (52). Significant efforts have been made to stratify patients prior to treatment on the basis of circulating cytokine, IgE, and eosinophil levels. One population-based study reported that approximately 80% of the severe asthma population of the United States is eligible for at least one biologic therapy based on current Food and Drug Administration (FDA) guidelines (1). Importantly, despite significant clinical interest in similar targets, no biologic therapies have been approved for EoE.

Mepolizumab and Benralizumab

There is a promising safety profile for long-term IL-5 and IL-5R blockade (53); however, results in terms of symptom reduction are heterogeneous, and challenges persist in identifying patients whose disease is likely to respond to IL-5 blockade (5). Mepolizumab treatment led to a significant reduction in flare exacerbations in patients with hypereosinophilic syndrome (HES) (2) and attenuated symptoms in patients with severe eosinophilic asthma with nasal polyposis (54). The best predictor of response to mepolizumab in patients with severe eosinophilic asthma remains high peripheral blood eosinophil counts at the start of treatment (54). Decreased circulating and tissue eosinophil counts have been observed in multiple trials using anti-IL-5 or anti-IL-5R; however, it has been repeatedly observed that decreasing eosinophil counts is not uniformly accompanied by attenuated disease symptoms or exacerbations (6, 55, 56).

Moreover, serum levels of CCL-13, CCL-17, CCL-22, and eotaxin-1 were increased in patients treated with mepolizumab, pointing to potential compensatory inflammatory mechanisms in patients treated with IL-5 blockade (3).

Omalizumab

The role of IgE in type 2 inflammation and its responsiveness to therapeutic targeting has received much attention (49, 57, 58). Omalizumab blunted increased blood eosinophilia in patients with allergic asthma who were exposed to rhinovirus and was associated with decreased respiratory symptoms (57). In chronic rhinosinusitis with nasal polyps (CRSwNP), omalizumab was associated with decreases in T and B cell activation, inflammatory cytokine release, and symptom scores (59).

Lirentelimab

Siglec-8 is expressed primarily on eosinophils and mast cells, and its ligation on eosinophils results in apoptosis (60-62). Antibody targeting of Siglec-8 with Lirentelimab (AK002) depleted eosinophils and blocked mast cell activation in preclinical murine models of allergic and non-allergic inflammation (63, 64). Importantly, intravenous lirentelimab attenuated symptoms in patients with eosinophilic duodenitis (EoD) and EoG and had a promising safety profile compared with placebo (36).

Cendakimab

Although eosinophils do not express the IL-13 receptor and are not direct targets of IL-13 signaling, epithelial cells secrete eotaxin and other inflammatory mediators following IL-13-stimulation; therefore, IL-13 is a target for treating eosinophilic diseases (65). MicroRNA 1 (miR-1) is downregulated in response to IL-13 signaling, making it a potential in vivo target of IL-13 (66). miR-1 expression in endothelial cells blocks expression of eotaxin and thymic stromal

lymphopoietin (TSLP) and is inversely correlated with airway eosinophils in asthma (66). Neutralizing IL-13 was investigated in an adult cohort of patients of EoE and demonstrated histological and clinical benefit in a phase 2 double blind study followed by an open-label extension (26, 67, 68). Compared with placebo, the higher dose of neutralizing antibody led to a significant decrease in esophageal eosinophils as well as E-cadherin and vimentin staining on esophageal epithelial cells, which was associated with epithelial-to-mesenchymal transition and fibrosis in patients with EoE (26). As *IL13* expression is heterogeneous in active EoE, the stratification of patients prior to IL-13–targeting treatment is an area of ongoing investigation (16).

Glucocorticosteroids

Glucocorticosteroids lead to global immune suppression, cause rapid eosinophil apoptosis, and have been used to treat eosinophilic diseases. To attenuate off-target effects of systemic steroids, treatment strategies have pursued local administration. Recently, novel formulations of glucocorticosteroids specialized for mucosal deposition in the esophagus have demonstrated efficacy in EoE (11, 48).

EOSINOPHIL STUDIES IN DISTINCT TISSUES

Remarkable progress has been made recently toward understanding how eosinophil functions specialize in unique tissue environments and how mechanisms identified in specific diseases may apply to diseases in other similar tissues (23).

Blood

Circulating eosinophils may predict their activation state in tissues. Moreover, there is prognostic potential for their phenotypes in the blood due to the relative ease of obtaining blood compared with tissue biopsies. Surface expression of the activation and adhesion marker CD41L was increased on circulating eosinophils of patients with EoE and positively correlated with eosinophil inflammation in the esophagus (69). Similarly, surface-bound SEMA4D was decreased on circulating eosinophils in patients with CRSwNP, leading to increased soluble SEMA4D in serum and in nasal polyp tissue biopsies (70). Moreover, disease was attenuated in a murine model of CRS using SEMA4D-deficient mice, and neutralizing SEMA4D reversed clinical phenotypes of eosinophilic chronic rhinosinusitis, including eosinophil infiltration (70). These results illustrate important features of eosinophil activation in the circulation that precede tissue-resident phenotypes.

Several studies have examined correlations between circulating eosinophil counts and exacerbations of eosinophilic diseases. In chronic spontaneous urticaria, peripheral eosinopenia is observed in patients in conjunction with significant eosinophil recruitment to tissue (71, 72). A recent preliminary study has shown benefit of chronic spontaneous urticaria by treatment with benralizumab, which depleted eosinophils (73). Conversely, peripheral eosinophilia in pediatric cases of severe asthma predicted resolution over 3 years of follow-up (74). Eosinophilia was observed in patients with allergic asthma, but not control individuals, following rhinovirus infection; correlated with respiratory symptoms; and was attenuated by systemic IgE depletion

(57). Furthermore, serum IL-6 and expression of its receptor positively correlated with asthma severity but were independent of circulating or tissue eosinophil levels or other markers of type 2 immunity (75, 76). The fact that peripheral eosinophilia correlated with some –but not all – clinical manifestations of eosinophilic disease is further evidence of the range of eosinophil functions and their contribution to disease.

Lung and Upper Airway

There has been significant interest in understanding eosinophil recruitment and activation to lung, sputum, and upper airway in asthma and chronic rhinosinusitis. Reports from working groups focused on phenotyping and tracking the progression of severe asthma reported that high eosinophil counts in the sputum at baseline correlated with high exhaled nitric oxide, high serum IgE, and elevated eosinophils in the circulation (77). Although eosinophil levels in sputum tended to decrease longitudinally, patients who initially presented with the highest sputum eosinophil levels continued to have higher eosinophils than did other patients. These results support a paradigm wherein eosinophils participate in the inflammatory and resolving phases of disease.

Eosinophilic inflammation is canonically associated with CRSwNP, and the diagnosis and quantification of eosinophils in nasal polyp biopsies is of clinical interest as it predicts recurrence and response to treatment (78). T helper type 2 (Th2) cytokines acting on epithelial cells to suppress the secretion of anti-inflammatory cytokines has been associated with eosinophilic inflammation in CRSwNP (79). An elegant study recently reported decreased expression of stimulator of interferon genes (STING) exclusively in eosinophilic polyps from patients with CRSwNP but not in non-eosinophilic polyps or biopsies from control individuals (65). The authors identified epithelial cells as the source of STING tissue expression and reported that the

type 2 cytokines IL-4 and IL-13 downregulated STING expression, resulting in a shift from interferon to eotaxin expression (65).

Gene and protein expression patterns have identified multiple endotypes of CRS without nasal polyps (CRSsNP), including a “T2” endotype marked by Th2 cytokines and IgE-dependent eosinophil activation (80). It has recently been appreciated that approximately half of individuals with CRSsNP exhibit a Th2 inflammatory signature and eosinophilic inflammation (81). This result highlights the importance of understanding the pleiotropic functions of eosinophils and their contributions to distinct disease states.

INTERACTIONS WITH IMMUNE CELL TYPES

Mast Cells and Basophils

Interactions between mast cells and eosinophils have received significant attention recently in conditions including atopic dermatitis, EoE, chronic spontaneous urticaria, and CRS (72, 82, 83). Mechanisms of eosinophil activation in association with mast cell activation have been studied as drivers of pathology in atopic dermatitis, chronic spontaneous urticaria, and other allergic disorders (84). Efforts to stratify patients with atopic dermatitis are ongoing, with reported biomarker analysis demonstrating promising results based on CCL17 and the resulting Th2 infiltration (85). Changes to the T helper cell landscape were observed in a cohort of patients with atopic dermatitis treated with dupilumab, specifically a shift away from eosinophil-associated Th2 in favor of Th17 (86). The multifaceted nature of immune interactions and relative contributions of eosinophils to inflammation in the skin is an area of active investigation.

Transcriptional detection of mast cell and basophil markers in sputum of individuals with severe asthma was correlated with elevated eosinophils in the airway and in blood (87). In addition to decreasing circulating and tissue eosinophil numbers, mepolizumab treatment associated with decreased circulating basophils and markers of mast cell inflammation in aspirin-exacerbated respiratory disease (7). Given their joint involvement in multiple inflammatory diseases, the relative contributions of eosinophils and mast cells in inflammatory diseases is an area of active ongoing investigation.

Neutrophils

Correlations between neutrophilic and eosinophilic responses have been a subject of interest, particularly in pulmonary inflammatory pathologies, including asthma and CRSwNP. In a multi-site study of adult severe asthma, a significant correlation was observed between neutrophil and eosinophil numbers in sputum, pointing to an important role of intercellular crosstalk and shared

inflammatory signals (77). A study of patients with severe eosinophilic asthma treated with benralizumab identified four groups of patients with different transcriptional profiles and treatment responsiveness (6). Importantly, this study identified increased neutrophilic inflammation in patients who required corticosteroids in addition to benralizumab to control symptoms (6). Neutrophil infiltration was reported as a feature of eosinophilic CRSwNP, and neutrophil activation and extracellular traps were reported in the presence of bacterial colonization (88). The relative contributions of eosinophils and neutrophils in inflammatory diseases is of particular interest in terms of delineating eosinophilic pathologies, especially as they share many activation phenotypes.

Lymphocytes

Lymphocytes, including T cells, B cells, and innate lymphoid cells (ILCs), secrete cytokines that activate and chemoattract eosinophils. Polarized Th2 cells are the source of type 2 cytokines in many inflammatory diseases (81, 82, 86). Tissue-resident Th2 cells and a chemoattractant CCL17 showed promise for the identification and stratification of patients with atopic dermatitis (85). ILC2 cells were recently identified as the source of type 2 cytokines in nasal polyps (82) and produced IL-5 in response to signals from IgE-sensitized mast cells (89). B cells secrete IgA, IgE, and IgG, which have a significant role in inflammation and tolerance in the mucosal environment and are critical to the development of food allergic responses (90). Antigen-bound antibodies recognized by Fc receptors trigger secretion of inflammatory mediators that recruit and activate eosinophils. The myriad mechanisms by which T cells, B cells, and ILCs may activate eosinophils are well characterized, and are targeted by many therapeutic strategies currently being investigated for eosinophilic diseases.

CURRENT METHODOLOGIES

Next-Generation Sequencing

The ever-increasing menu of next-generation sequencing technologies that generate whole-genome transcriptional data and provide information about transcription factor binding and promoter activity are powerful tools for understanding complex pathologies. Elegantly coordinated RNAseq and chromatin immunoprecipitation sequencing (ChIPseq) experiments using esophageal epithelial cells demonstrated the cumulative response of esophageal epithelial cells to IL-13 stimulation and furthermore demonstrated the kinetic response wherein *ANO1* was among the first genes to be strongly upregulated upon IL-13 treatment, leading to the hyperproliferative phenotype associated with tissue pathology in EoE (25). RNAseq of tissue biopsies was used to identify a diagnostic transcriptional signature in EoE, and the authors identified associations between gene expression and pathologic features that offered robust insight into disease progression (10).

Multiple foundational studies have recently reported transcriptional and epigenetic features of eosinophils, which greatly improve understanding of eosinophilic diseases. The transcription factor Aiolos (*IKZF3*) has a critical role in regulation of intracellular signaling and chemotaxis in both murine and human eosinophils (91). In the context of growing interest in the role and regulation of long-lived tissue-resident eosinophils, it was reported that eosinophils differentially express the majority (58%) of their transcriptome after 7 days of differentiation in the presence of IL-5 or in an in vitro coculture model of EoE (30). The epigenetic landscape of eosinophils includes enhancer elements that support specialization, which were identified by shared ATAC, H3K4me1, and H3K27ac peaks (92). Importantly, allergic disease susceptibility loci were associated with eosinophil-specific superenhancers (92). Increased understanding of genetic changes that occur during eosinophil maturation and specialization is critical for ongoing efforts to diagnose and treat eosinophilic disease.

Single-cell RNAseq (scRNAseq) of EoE biopsy tissue has identified the cell type-specific expression of genes that are strongly associated with human disease susceptibility or pathogenesis, such as *CAPN14* and *SAMM50*, which are predominantly expressed by esophageal epithelial cells, and *GATA3* and *BACH2*, which are expressed by lymphocytes (93). Challenges remain in obtaining scRNAseq data on human eosinophils because they are not reliably detected in patient samples from tissues with heavy eosinophil infiltration (53, 94, 95). There is ongoing interest in the development of increasingly robust technologies that would bring scRNAseq to bear on human eosinophils, and one group has reported an eosinophil signature marked by TNF α signaling in scRNAseq data from human EoE biopsies (19). Ongoing work will validate and expand on this work in EoE and other eosinophilic diseases.

Confocal Microscopy

High-resolution microscopy has been increasingly leveraged to understand eosinophil differentiation, activation, and degranulation. In addition to demonstrating rapid colocalization of Charcot-Leyden crystals (CLC) with CD63 associated with secondary granules in response to IFN γ , high-resolution imaging indicated the presence of large, empty granules in human eosinophils differentiated from cord blood progenitor cells transfected with short hairpin RNA against *CLC* (96). There is particular interest in CLC since its crystals drive type 2 immunity and allergy (97).

Increasingly, machine learning and artificial intelligence are being leveraged to analyze pathologic specimens (26, 78, 98). The benefits of such an approach include standardization across multiple investigators and study sites and potentially leveraging the strengths of machine learning to identify patterns and subtleties that may be missed by human eyes.

Proteomics and Metabolipidomics

Decellularized extracellular matrix from fibrotic esophageal tissue in EoE was analyzed by mass spectrometry, resulting in the identification of specific components of the profibrotic extracellular matrix that may respond to therapeutic targeting (99). Similarly, proteomic analysis of nasal mucus samples was used to identify changes in immune, antioxidant, and tissue remodeling pathways in chronic rhinosinusitis (100). Mass spectrometry has also been used to identify endogenous Siglec-8 ligands in epithelial cells to gain mechanistic insight into the control of eosinophil activation in vivo (101). In addition to proteomic analysis, mass spectrometry-based methods have been employed to quantify bioactive molecules with pro-resolving function in the inflamed lung; for instance, it was found that maresin conjugates in tissue regeneration (MCTRs), which are more abundant in normal vs. asthmatic lungs, antagonize leukotriene D4 to attenuate airway contractility and eosinophil recruitment (33). Collectively, studies using mass-spectrometry add critical information to the understanding of pathological tissue states.

Genome-wide Association Studies (GWAS)

Genetic associations have been of particular interest in rare allergic diseases, such as EoE, for which genetic drivers have been implicated in mechanisms of disease (93, 102, 103). Recently, highly-powered studies of multiple cohorts of EoE patients identified novel genetic susceptibility loci, some of which support shared mechanistic and genetic features with atopic conditions including childhood-onset asthma (103). By combining GWAS with RNAseq of tissue biopsies, researchers identified differential expression of EoE-associated genes in patients with EoE compared with controls (102). Furthermore, patterns in cell type-specific expression of disease-associated genes resulted in insight into mechanistic underpinnings of susceptibility. These studies offer important insight into diseases with marked heritability but whose genetic underpinnings have previously been elusive, and illuminate therapeutic targets for EoE and atopic disorders with shared mechanistic drivers.

CONCLUDING REMARKS

There have been many exciting recent advances toward understanding key functions of eosinophils and their role in disease, and ongoing studies are poised to make major strides toward the understanding of eosinophilic inflammation (Table III). Eosinophil depletion in humans is clinically beneficial in a number of diseases, and “human eosinophil knockouts”, generated by these drugs, do not demonstrate any major deficits, indicating that many if not all eosinophil functions may be actualized by redundant activities of other myeloid cell types (53). Many cutting edge technologies are currently being leveraged to uncover mechanisms of eosinophilic diseases, and are poised to identify novel therapeutic targets to deplete and regulate eosinophils in health and disease.

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733 2021.
734

735 **Tables****Table I: Key Advances in EGID in 2021**

- Involvement of eosinophils in normal-like and fibrostenotic as well as inflammatory phases of EoE(16).
- ANO1 as a target of IL-13 and driver of inflammation in EoE (25).
- Serum biomarker score, including eotaxin-3 and IL-5, detects EoG (10).
- Efficacy of biologics targeting IL-13 (26, 67, 68), IL-4 (51), IL-5 (53), and Siglec-8 (36, 63, 64) in treatment of EoE and EoG.

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Table II: Recent GWAS findings in EoE (102, 103).	
Validated GWAS hits	2p23 (CAPN14*)
	5q22 (TSLP*/WDR36*†)
	11q13 (LRRC32*/EMSY*)
	12q13 (STAT6)
	19q13 (ANKRD27)
	16p13 (CLEC16A*)
New GWAS hits	2q12 (TMEM182)
	5q31 (RAD50)
	6p22 (SOX4)
	8q22 (MATN2)
	10q21 (PRKG1)
	11p15 (RHOG)
	11q13 (SHANK2)
	13q12 (GPR12)
	15q22 (RORA)
	15q23 (SMAD3)
New GWAS hits – Meta-Analysis	1p31
	5q23
	6q15 (BACH2 [†])
	6q21(SOBP)
	8p21 (TNFRSF10B[†]/C[†])
	10p14 (ITIH5/GATA3 [†])
	17q12 (ARHGAP23)
	18q12 (GALNT1)
	22q13 (PNPLA3 [†])
Bold genes are upregulated in EoE vs. control biopsies. * Genes expressed in esophageal tissue/epithelium. † Genes expressed in leukocytes.	

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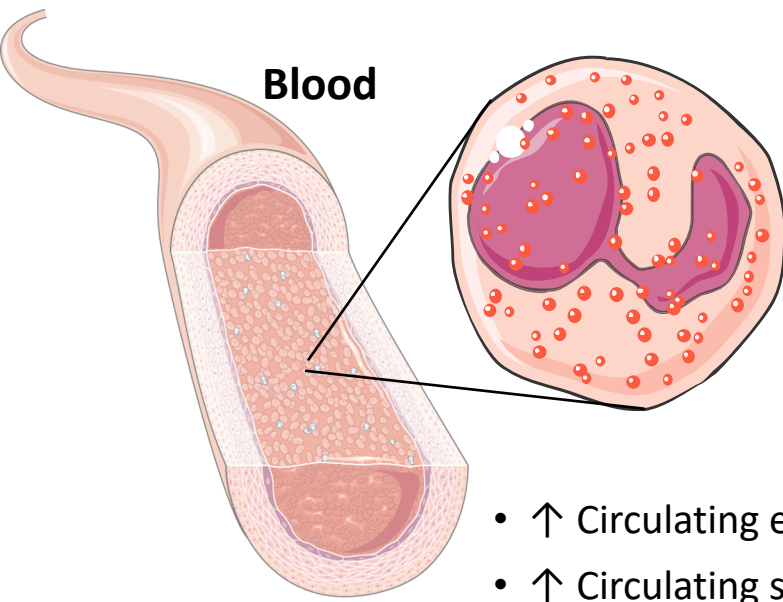
Table III: Important areas of ongoing eosinophil research.

- Describe distinct pro-inflammatory and pro-regulatory eosinophil functions in human disease.
- Identify shared mechanisms of pathology in atopic, respiratory, and digestive eosinophilic diseases.
- Correlate unique eosinophil phenotypes in tissue and blood to improve prognostic value of blood-based measurements.
- Advance methodologies to enable single-cell transcriptional and epigenetic analysis of human eosinophils.

739

740 **Figure 1: Recent advances in tissue-specific eosinophil specialization.**

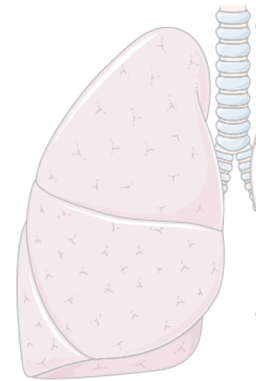
Journal Pre-proof



Blood

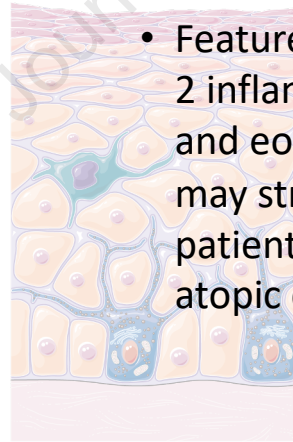
- ↑ Surface CD41L on circulating eosinophils in EoE.
- ↓ Surface SEMA4D on circulating eosinophils in CRSwNP.
- Aiolos regulates CCR3-dependent eosinophil chemotaxis.
- ↑ Circulating eotaxin-3 and IL-5 as biomarkers in EoG.
- ↑ Circulating soluble SEMA4D in CRSwNP.

Lung & Upper Airway



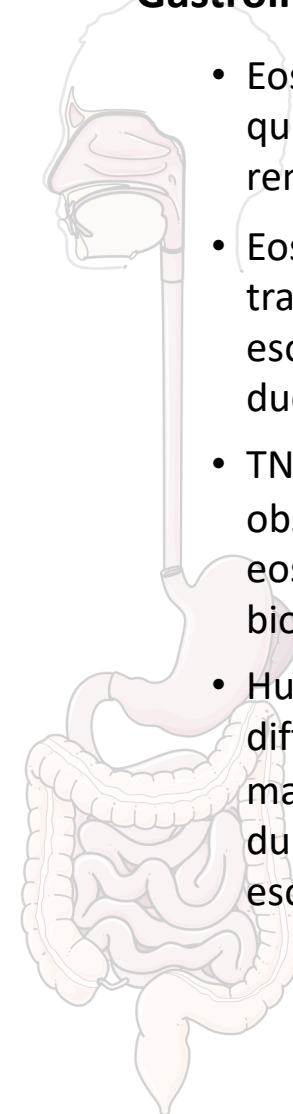
- Sputum eosinophil counts correlate with exhaled NO• and serum IgE.
- Type 2 inflammatory cytokines shift gene expression from interferon to eotaxin in eosinophilic nasal polyps.
- Eosinophilic inflammation occurs in >40% of CRS without nasal polyps.
- Novel endogenous Siglec-8 ligand identified in human airway epithelial tissue.

Epidermis



- Features of type 2 inflammation and eosinophilia may stratify patients with atopic dermatitis.

Gastrointestinal Tract



- Eosinophils are present during quiescent, inflammatory, and remodeling phases of EoE.
- Eosinophils exhibit differential transcription factor usage in esophageal compared with duodenal tissue.
- TNF α -signaling signature observed by scRNAseq in eosinophils from esophageal biopsies in active EoE.
- Human eosinophils differentially express the majority of their transcriptome during differentiation with esophageal epithelial cells.