Advances in allergy, asthma, and immunology

Advances in mechanisms of allergic disease in 2016

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This review highlights advances in mechanisms of allergic disease, particularly type 2 innate lymphoid cells; T_H2 lymphocytes; eicosanoid regulation of inflammation; extracellular vesicles in allergic responses; IL-33; microbiome properties, especially as they relate to mucosal barrier function; and a series of findings concerning the allergic inflammatory cells eosinophils, basophils, and mast cells. During the last year, mechanistic advances occurred in understanding type 2 innate lymphoid cells, particularly related to their response to ozone, involvement with experimental food allergy responses, and regulation by IL-33. Novel ways of regulating T_H2 cells through epigenetic regulation of GATA-3 through sirtuin-1, a class III histone deacetylase, were published. The understanding of eicosanoid regulation of inflammation increased and focused on additional properties of phospholipase A_2 and the role of prostaglandin D_2 and its receptors and inhibitory prostaglandin E_2 pathways. Mechanisms through which extracellular vesicles are released and contribute to allergic responses were reported. There was a deeper appreciation of mucosal barrier function, the epithelial alarmin IL-33, and the microbiome. Finally, there were advances concerning allergic inflammatory cells (mast cells, basophils, and eosinophils) that will undoubtedly have an effect on disease understanding and new therapeutic strategies. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Allergy, immunology, innate, inflammation, food allergy, asthma, mast cell, eosinophil, ozone, IL-33, extracellular vesicles*

Abbreviations used

AERD:	Aspirin-exacerbated respiratory disease
CRTH2:	Chemoattractant receptor–homologous molecule expressed on T_H2 cells
DP1:	Prostaglandin D_2 receptor 1
DP2:	Prostaglandin D_2 receptor 2
DUOX1:	NADPH oxidase dual oxidase 1
EET:	Eosinophil extracellular trap
EGPA:	Eosinophilic granulomatosis with polyangiitis
EoE:	Eosinophilic esophagitis
EP:	E prostanoid receptor
EV:	Extracellular vesicle
hPGDS:	Hematopoietic prostaglandin D synthase
ILC:	Innate lymphoid cell
ILC2:	Type 2 innate lymphoid cell
ILC3:	Type 3 innate lymphoid cell
IL-1R1:	IL-1 type 1 receptor
IL-4R:	IL-4 receptor
LTD_4:	Leukotriene D_4
LXA_4:	Lipoxin A_4
miR:	MicroRNA
MRGPRX2:	Mas-related G protein–coupled receptor X2
MyD88:	Myeloid differentiation primary response gene 88
peT_H2:	Pathogenic effector T_H2
PGD_2:	Prostaglandin D_2
PGE_2:	Prostaglandin E_2
PLZF:	Promyelocytic leukemia zinc finger
PPI:	Proton pump inhibitor
REE:	Responsive esophageal eosinophilia
RSV:	Respiratory syncytial virus
sPLA2-X:	Secreted phospholipase A_2 group X
STAT6:	Signal transducer and activator of transcription 6
TSLP:	Thymic stromal lymphopoietin

This review highlights advances in mechanisms of type 2 innate lymphoid cells (ILC2s); $CD4^+$ T_H2 lymphocytes; eicosanoid regulation of inflammation; extracellular vesicles (EVs) in allergic responses; IL-33; microbiome properties, especially as they relate to mucosal barrier function; and allergic the inflammatory cells eosinophils, basophils, and mast cells. Although these topics have been widely covered in the literature, this review focuses on articles published in the *Journal of Allergy and Clinical Immunology* in 2016. We hope that the reader will find this summary article a useful supplement to reading the original articles.

ILC2s

There continues to be an increase in knowledge of ILC2s in different disease states. Over the past year, the role of ILC2s in inflammation induced by ozone, obesity, asthma, food allergy, and respiratory syncytial virus (RSV) infection has been described. In addition, the factors that regulate the development

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Disclosure of potential conflict of interest: M. E. Rothenberg has received grants from the National Institutes of Health, a US-Israel Binational Grant, and the Patient-Centered Outcomes Research Institute; has consultant arrangements with Celgene, Genetech, Immune Pharmaceuticals, NKT Therapeutics, and PulmOne Therapeutics; has received payment for lectures from Merck; is the inventor on a patent owned by Cincinnati Children's Hospital Medical Center; and has stock/stock options with PulmOne Therapeutics, NKT Therapeutics, and Immune Pharmaceuticals. H. Saito has received payment for lectures from Shiseido, Merck Sharp and Dohme KK, and AstraZeneca. R. S. Peebles declares that he has no relevant conflicts of interest.

Received for publication July 10, 2017; revised August 25, 2017; accepted for publication August 25, 2017.

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0091-6749/$36.00

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https://doi.org/10.1016/j.jaci.2017.08.029

of ILC2s have been elucidated further, and the enzyme responsible for IL-33 release that leads to ILC2 stimulation has been identified.

In a recent article, ozone exposure resulted in release of IL-33 in the airways of both BALB/c and C57BL/6 mice, yet ILC2 numbers were increased only in BALB/c but not C57BL/6 mice after ozone challenge.[1] ILC2 depletion with anti-Thy1.2 antibody administration blocked ozone-induced airway responsiveness in BALB/c mice, and adding back ILC2s restored ozone-induced airway responsiveness. The authors suggested intrinsic differences in ILC2s between BALB/c and C57BL/6 mice.

In another article, evidence that innate lymphoid cells (ILCs) exacerbate airway disease in the setting of obesity was reported. In the setting of diet-induced obesity in mice, there were an increased number of ILC2s and type 3 innate lymphoid cells (ILC3s) compared with lean mice, although there was no increase in airway responsiveness.[2] When allergic airway inflammation was induced with house dust mite allergen, obese mice had greater numbers of ILC2s and ILC3s than lean mice, and this was associated with greater lung IL-33 and IL-1β protein expression in obese mice. Anti-CD90–mediated ILC depletion reduced allergic airway inflammation and recruitment of T_H2 and T_H17 cells.[2]

In a cross-sectional study of patients with severe eosinophilic or mild atopic asthma, patients with severe asthma had significantly greater numbers of total and type 2 cytokine–producing ILC2s in the blood and sputum, as determined by means of intracellular cytokine staining with flow cytometric analysis, than did patients with mild asthma.[3] Also, there was a greater number of IL-5^+IL-13^+ ILC2s in the sputum of patients with severe asthma and airway eosinophils of greater than 3% than in that of patients with a lower percentage of eosinophils. Interestingly, there was no difference in intracellular cytokine expression by CD4 cells and eosinophilopoietic progenitor cells in blood or sputum between the groups with severe and mild asthma.

New evidence from mouse studies suggests that ILC2s can promote food allergy. Mice that express a gain-of-function mutation in the IL-4 receptor (IL-4R) α chain were shown to be predisposed to food allergy and have an increased number of ILC2s, which secrete IL-4 as a result of IL-33 stimulation.[4] IL-4 was shown to inhibit generation of allergen-specific regulatory T cells and augment the development of food allergy. This raises the possibility that antagonizing IL-33 can reduce food allergy.

The mechanisms leading to IL-33 release and the subsequent stimulation of ILC2 responses were defined.[5] The NADPH oxidase dual oxidase 1 (DUOX1) was shown to be critical for IL-33 release from cultured human or mouse epithelial cells in response to challenge with allergen extracts from either *Alternaria alternata* or *Dermatophagoides pteronyssinus*. Allergen-induced IL-33 secretion by epithelial cells was calcium dependent and mediated by epidermal growth factor receptor signaling and involved cysteine oxidation. The clinical relevance of these results was supported by an increase in DUOX1 expression in nasal epithelial cells from asthmatic patients.

In another article, IL-25 was shown to promote allergic inflammatory responses in that systemic administration or overexpression of IL-25 augmented production of T_H2 cytokines and eotaxin. Mice that overexpressed IL-25 in the intestine and were sensitized to ovalbumin had a significantly greater number of IL-5– and IL-13–expressing ILC2s associated with anaphylactic responses after just 4 ovalbumin challenges than did sensitized and challenged nontransgenic animals.[6] There was an interaction between ILC2 and antigen-specific T_H2 cell numbers in that T_H2 cells enhanced ILC2 function in response to IL-25, whereas ILC2s did not promote experimental food allergy in signal transducer and activator of transcription 6 (STAT6)–deficient mice that lacked the capacity to generate T_H2 cells.

Thymic stromal lymphopoietin (TSLP) was also shown to regulate ILC2 production of IL-13 in the setting of RSV infection. In a mouse model of RSV infection with RSV 01/2-20, a strain that results in a mixed T_H2/T_H1 host immune response, TSLP antagonism significantly reduced IL-13–producing ILC2s, airway responsiveness, and mucus metaplasia and prevented RSV-induced weight loss without affecting viral load.[7] TSLP antagonism during infection with 2 other RSV strains that induced T_H2 responses produced similar results. These results suggest that targeting TSLP might be a potential therapeutic target during severe RSV infection.

Expression of the transcription factor promyelocytic leukemia zinc finger (PLZF) at the innate lymphoid precursor stage was shown to have a long-lasting effect on the functionality of mature ILC2s, which seems to be mediated mainly by stimuli associated with innate immunity.[8] Mice genetically deficient in PLZF had a defect in IL-5 and IL-13 secretion in response to papain, IL-25, or IL-33. As a result, $PLZF^{-/-}$ mice had decreased recruitment of eosinophils and a reduction in mucus metaplasia compared with wild-type mice. In contrast, $PLZF^{-/-}$ mice had no defect in the ability to generate allergic inflammation to ovalbumin sensitization and challenge, which is mediated by $CD4^+$ T cells and the adaptive immune response.

In another study, Lombardi et al[9] found that the frequency of ILC2s and ILC3s in PBMCs was the same between allergic subjects (allergic rhinoconjunctivitis) and nonallergic subjects. However, they found a difference when they focused only on patients with allergic rhinoconjunctivitis. In particular, patients with allergic rhinoconjunctivitis and asthma had more ILC2s and ILC3s in the blood than did patients with allergic rhinoconjunctivitis without asthma. However, the frequency of ILC2s and ILC3s in blood was the same between healthy subjects and patients with allergic rhinoconjunctivitis with asthma. There were greater numbers of ILC2s and ILC3s in the peripheral blood of asthmatic patients compared with nonasthmatic subjects, and type 1 ILCs from patients with chronic rhinoconjunctivitis produced decreased IFN-γ levels compared with those from healthy control subjects. ILC2s from asthmatic patients had increased gene expression of the proto-oncogene c-Fos, the protein Fos B, and JUN, protein products that compose the transcription factor activator protein 1. However, ILC2s from subjects without allergic disease had greater gene expression of NKG7, suppressor of cytokine signaling 1, and TBX21, proteins that inhibit T_H2 transcriptional programs. A summary of ILC advances published recently in the *Journal* is shown in Table I.

$CD4^+$ T_H2 CELLS

GATA-3 is the master regulator of T_H2 differentiation and might be negatively regulated by sirtuin-1, a class III histone deacetylase.[10] PBMCs obtained from healthy subjects were treated with sirtinol, a specific inhibitor of sirtuin-1 and sirtuin-2, with a resultant increase in *IL4* transcripts and

TABLE I. Recent *Journal* advances concerning innate lymphoid cells

- Intrinsic differences between murine ILCs from BALB/c and C57BL/6 mice were identified.
- ILCs exacerbate airway disease in the setting of obesity.
- ILC2s can promote food allergy.
- A high-fat diet has been shown to exacerbate allergic airway inflammation characterized by infiltration of both ILC2s and ILC3s into the lung.

acetylation of GATA-3. The clinical relevance of these results was supported by the finding that the deacetylase activity of the NAD-dependent protein deacetylase sirtuin-1 was decreased in PBMCs from patients with severe asthma compared with those from either patients with nonsevere asthma or healthy control subjects.

There is evidence that sirtuin-6, a histone deacetylase and mono-ADP ribosyltransferase enzyme functionally involved in multiple molecular pathways related to aging, including DNA repair, telomere maintenance, glycolysis, and inflammation, can also suppress allergic airway inflammation by means of deacetylation of GATA-3. Tracheally instilling adenoviruses expressing sirtuin-6 into mice before ovalbumin challenge significantly reduced lung inflammatory cells and airway mucus.[11] Although GATA-3 acetylation was enhanced by ovalbumin sensitization and challenge, sirtuin-6 overexpression significantly decreased GATA-3 expression. Therefore 2 different sirtuins inhibit GATA-3 activity and potentially could be therapeutic targets in patients with allergic airway disease.

T_H2 cell production of IL-13 was enhanced when IL-1β was present during sensitization of mice to house dust mite allergen, resulting in increased peribronchial infiltrates, mucus production, and goblet cell metaplasia.[12] In contrast, T_H2 priming in the presence of IL-1β antagonism with anakinra resulted in lower expression of IL-13, which blunted cellular infiltration and mucus. These results suggest that signaling through the IL-1 receptor might be an important factor in the differential effector function of T_H2 cells.

The role of $CD4^+$ T cell–derived IL-4 and IL-13 in the murine asthma phenotype was more thoroughly described when IL-4 deficiency did not block the onset of T_H2 immune responses, airways responsiveness, or mucus metaplasia.[13] However, IL-13 deficiency inhibited development of the pathophysiologic features characteristic of asthma mentioned above. Signaling through IL-4Rα, a receptor subunit shared between IL-4 and IL-13, was critical to the asthma phenotype, and the absence of IL-4Rα signaling promoted T_H17 responses. It is notable that the humanized anti–IL-4Ra antibody dupilumab, which is now US Food and Drug Administration approved for atopic dermatitis, has been shown recently to be effective for human asthma.[14-16]

IL-25 and IL-33 likely have a role in the pathogenesis of chronic rhinosinusitis with nasal polyposis. In nasal polyp tissue from patients with chronic rhinosinusitis with nasal polyposis, there was a large population of T_H2 cells that coexpressed the IL-25 receptor subunit IL-17 receptor B and expressed mRNA for the transmembrane and soluble forms of the IL-33 receptor subunit ST2. Indeed, T_H2 cells are activated by both IL-25 and IL-33, with resultant increased production of IL-5 and IL-13.[17] In IL-17 receptor B–positive $CD4^+$ cells, several identical sequences of the T-lymphocyte receptor variable β-chain complementary-determining region 3 were identified in different subjects, suggesting the possibility of clonal expansion by a common antigen.

Our understanding of the metabolic pathways that drive T_H2 polarization is in its infancy. RhoA is essential for coupling glycolysis to T_H2 polarization by mediating IL-4R RNA expression and T_H2-specific signaling events.[18] Hyperactivation of RhoA promoted T_H2 polarization and induced allergic airway inflammation. In contrast, genetic deletion of RhoA blunted T_H2 *in vitro* and blocked the development of ovalbumin-induced allergic airway inflammation in mice while having no effect on T_H1 cell development. In addition, T_H2 cell development was shown to be inhibited by epicutaneous antigen sensitization in the presence of Toll-like receptor 9 agonist (CpG). The mechanism was dependent on shifting immunity to T_H1 and T_H17 responses through a myeloid differentiation primary response 88 (MyD88)–dependent response.[19]

EICOSANOID REGULATION OF INFLAMMATION

Phospholipase A_2 mediates the rate-limiting step in the production of arachidonic acid, the necessary substrate for generation of both prostaglandins and leukotrienes. The secreted phospholipase A_2 group X (sPLA2-X) was found in the endoplasmic reticulum, golgi, and granules of eosinophils.[20] Specific inhibition of sPLA2-X reduced N-formyl-L-methionyl-L-leucyl-L-phenylalanine–induced release of arachidonic acid and cysteinyl leukotriene formation by eosinophils. Short-term priming of eosinophils with IL-13 and TNF/IL-1β increased expression of the gene that encodes sPLA2-X. These results reveal that sPLA2-X has an important function in cysteinyl leukotriene production by eosinophils and could have a role in the treatment of diseases in which eosinophil-derived leukotrienes have a pathogenic role.

Prostaglandin E_2 (PGE_2) is an important brake on the generation of leukotrienes in patients with aspirin-exacerbated respiratory diseases (AERD).[21] Using fibroblasts obtained from the nasal mucosa of control subjects or polyps of patients with AERD, the expression of PGE_2-related proteins was assessed. This included analysis of enzymes involved in PGE_2 synthesis, COX-2, and microsomal prostaglandin E synthase 1; the receptor of a cytokine that induces COX-2, IL-1 type 1 receptor (IL-1R1); and E prostanoid receptor (EP) 2, a receptor through which PGE_2 signals to inhibit leukotriene production.[22] Basal expression of IL-1RI and EP2 was lower in the fibroblasts of polyps obtained from patients with AERD than fibroblasts obtained from the nasal mucosa of control subjects. Exogenous administration of either PGE_2 or selective EP2 agonists significantly increased expression of IL-1R1 by fibroblasts obtained from the nasal mucosa of control subjects, whereas there was minimal effect on fibroblasts of polyps obtained from patients with AERD. These data suggest that decreased expression of EP2 in patients with AERD can negatively regulate induction of IL-1R1, which in turn inhibits the ability of IL-1β to increase COX-2 and microsomal prostaglandin E synthase 1 expression, resulting in decreased PGE_2 production.

The biologic effects of PGE_2 are regulated by the receptors through which this prostaglandin signals. EP2 and EP4 are coupled to Gs, whereas EP3 is coupled to Gi. Leukotriene D_4 (LTD_4) and PGE_2 potentiated vascular permeability and resulting edema in mouse ears.[23] This effect was mediated by LTD_4

signaling through cysteinyl leukotriene receptor 1 and PGE_2 signaling through EP3, with resultant extracellular signal-regulated kinase and c-FOS phosphorylation, macrophage inflammatory protein 1β and COX-2 upregulation, and prostaglandin D_2 (PGD_2) production by mast cells. Concurrent treatment with cysteinyl leukotriene receptor 1 and EP3 antagonists inhibited vascular permeability, suggesting that targeting both of these receptors might blunt inflammation-induced vascular leakage.

Patients with AERD have increased levels of urinary cysteinyl leukotrienes that correlate with disease severity. Aspirin desensitization improves clinical symptoms, but the understanding of mechanisms through which desensitization improves outcome is incomplete. $CD3^+$ and $CD28^+$ activation of $CD4^+$ T cells results in increased STAT6 activation in patients with AERD compared with healthy subjects.[24] Treatment of $CD4^+$ T cells with the COX inhibitors indomethacin or aspirin before and during cellular stimulation decreased STAT6 activation in cells from subjects with and without AERD. However, the magnitude of the decrease in STAT6 activation was significantly greater in the cells of patients with AERD, providing one possible mechanism for why aspirin desensitization is effective in patients with AERD.

PGD_2 activates 2 G protein–coupled receptors: prostaglandin D_2 receptor 1 (DP1) and prostaglandin D_2 receptor 2 (DP2). PGD_2 activation of DP1, DP2, or both DP1 and DP2 on human macrophages *in vitro* induced TNF secretion and migration.[25] Subcutaneous administration of PGD_2 before airway LPS challenge enhanced neutrophilic lung injury, and this was mediated by signaling through both DP1 and DP2. Macrophage depletion reduced neutrophil function that had been heightened by PGD_2 pretreatment before LPS administration, highlighting the role of macrophages in this mouse model of lung injury. Inhibiting endogenous PGD_2 production blocked LPS-induced neutrophilic inflammation.

The PGD_2 receptor DP2 (also known as chemoattractant receptor–homologous molecule expressed on T_H2 cells [CRTH2]) has been identified as a marker for pathogenic effector T_H2 (peT_H2) cells, a T_H2 cell subpopulation with enhanced proinflammatory function.[26] In addition, those peT_H2 cells that express DP2 also express hematopoietic prostaglandin D synthase (hPGDS) and produce PGD_2, whereas conventional T_H2 cells do not. These $hPGDS^+$ cells expressed significantly greater levels of IL-5 and IL-13 than did $hPGDS^-$ T_H2 cells. The possible functional relevance of these peT_H2 cells was suggested by a high correlation with blood eosinophilia, and a greater number of these cells were found in the intestines of patients with eosinophilic gastrointestinal disease than healthy control subjects. In addition, peT_H2 cells preferentially expressed receptors for TSLP, IL-25, and IL-33 compared with conventional T_H2 cells, suggesting that they might have a heightened response or be activated by lower levels of these cytokines. It is notable that the CRTH2 antagonist fevipiprant was shown to reduce sputum eosinophilia in patients with severe eosinophilic asthma uncontrolled by high-dose corticosteroids.[27]

TSLP might be an important mediator of PGD_2 generation in patients with AERD. Whole nasal polyp *TSLP* mRNA strongly correlated with mRNA encoding hematopoietic PGD synthase and carboxypeptidase A_3, the latter of which is specific for mast cells.[28] Protein levels of the cleaved and active forms of TSLP were present in nasal polyps from patients with AERD, and recombinant TSLP induced PGD_2 production by cultured human mast cells. The association of PGD_2 with AERD was further confirmed by the finding that levels of stable urinary PGD_2 were 2-fold greater in patients with AERD compared with those in control subjects, and PGD_2 levels were increased subsequent to aspirin-induced reactions.

The possibility that IgE can be involved in AERD pathogenesis was supported by data that omalizumab reduced symptoms in patients with severe AERD who were treated for at least 12 months.[29] There was a reduction in urine levels of both leukotriene E_4 and the stable PGD_2 metabolite in 21 patients who were treated with omalizumab for asthma. A randomized, double-blind, placebo-controlled trial is necessary to confirm the role of omalizumab in treatment of AERD.

Lipoxins are eicosanoids derived from arachidonic acid that have anti-inflammatory and proresolution bioactivities. Lipoxin A_4 (LXA_4) blunted TNF-mediated neutrophil chemotaxis and activation while downregulating proinflammatory gene expression through blockade of the nuclear factor κB pathway.[30] On peripheral blood granulocytes from healthy control subjects and patients with intermittent asthma, LXA_4 significantly induced glucocorticoid receptor activity when combined with fluticasone compared with fluticasone alone or LXA_4 alone. The ability of LXA_4 to augment glucocorticoid receptor activity in the presence of a corticosteroid might explain part of the protective effect of lipoxins in asthmatic patients.

There is now evidence that eicosanoids might be involved in venom immunotherapy–associated tolerance. PBMCs stimulated with specific allergen produced increased PGE_2 and LXA_4 levels. Interestingly, there were no changes in the quantity of PGE_2 or LXA_4 over the course of venom immunotherapy.[31] The increase in PGE_2 and LXA_4 levels was also associated with production of IL-10 and TGF-β, which raises the possibility that PGE_2 and LXA_4 might have a role in the induction, development, and maintenance of tolerance that occurs in subjects who are administered venom immunotherapy.

EVs AND ALLERGY

EVs are a heterogeneous group of small membrane-coated vesicles with a diameter of 0.05 to 5 μm and can be classified into 3 groups (ie, exosomes, microvesicles, and apoptotic bodies), depending on their origins and characteristics (Fig 1). EVs released from the cellular membrane convey various DNAs, proteins, mRNAs, and microRNAs (miRs) and are considered to play an important role in allergic or immune responses, as has been well recognized in metastasis and progression of tumors. For example, in patients with sarcoidosis, more than 690 proteins were identified in EVs purified from bronchoalveolar lavage fluid, and in these patients several inflammation-associated proteins, such as leukotriene A_4 hydrolase and vitamin D–binding protein, were found to be upregulated.[32] Nasal epithelial cells express miR-146a, and the level of the miR is markedly lower in nasal epithelial cells of patients with nasal allergy than in healthy control subjects. Exposure to T_H2 cytokines suppresses miR-146a levels, which are contained in EVs and can be released from nasal epithelial cells. Nasal epithelial cell–derived miR-146a, which is contained in EVs, upregulated expression of IL-10 by monocytes.[33]

In addition, eosinophils can secrete EVs, and their secretion is increased in asthmatic patients.[34] Eosinophil extracellular traps

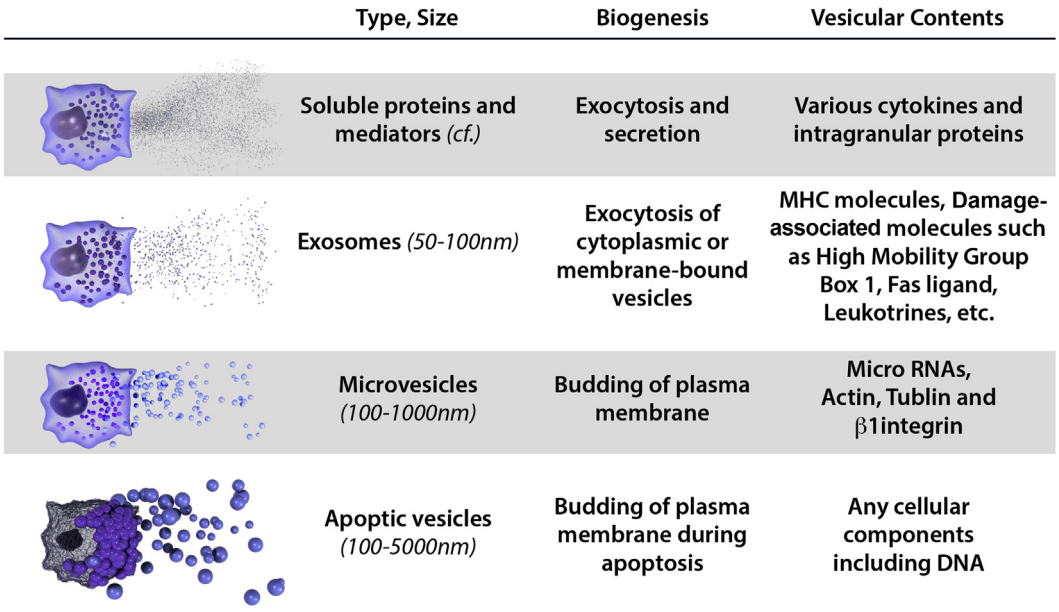


FIG 1. Type, size, and content of EVs.

(EETs) can be considered as a sort of EV. They are capable of killing bacteria by releasing nuclear and mitochondrial DNA in association with cytotoxic granule proteins.[35] EET formation was also found to be associated with increased IL-5 and periostin tissue levels and colonization with *Staphylococcus aureus* in patients with chronic rhinosinusitis with nasal polyps. EET formation was induced on exposure to *S aureus*, but not to *Staphylococcus epidermidis*, *in vitro*. Eosinophils were shown to migrate toward *S aureus* and entrap the bacteria both inside and outside mucosal tissue. In addition to generating EVs after eosinophil cytolysis, nonlysed eosinophils might also contribute to the release of EETs and other EVs.[35]

Platelet-derived EVs are released from the plasma membrane of platelets and are the most abundant EVs in human blood. Recent publications about the molecular and functional characteristics of platelet-derived EVs suggest that they have a key role in various processes, especially during inflammation, instead of being inert byproducts of cellular activation.[36] Regarding allergic inflammation, platelets express functional IL-33 protein, and papain-induced eosinophilic airway inflammation was attenuated by platelet depletion alone or followed by transplantation of platelets genetically lacking IL-33.[37] IL-33 is constitutively present in the nucleus and liberated during necrosis from cell types with abundant IL-33, such as epithelial cells. Unlike many other cytokines, IL-33 is not directly produced and released in response to the receptor-dependent signaling. However, necrosis is not the exclusive mechanism causing IL-33 release, especially regarding hematopoietic cell types, such as platelets or monocytes.[38] Biomechanical stress can also translocate IL-33 to membrane-bound secretory vesicles and triggers the passive and time-dependent release of IL-33 from living cells.[39]

IL-33: A VERSATILE CYTOKINE

IL-33 is now recognized to play a crucial role in inflammatory allergic diseases by triggering ILC2s to produce type 2 cytokines and by suppressing innate antiviral immunity.[40] It is widely known from genome-wide association studies that genetic variants of IL-33 and its receptor ST2 are associated with the risk of asthma onset. Ferreira et al[41] identified 4 novel asthma risk genes related to nucleotide synthesis and signaling by using a newly developed test of systematic analysis. Using an animal model of asthma, they demonstrated that agonists of the 2 gene products induced IL-33 release and subsequent eosinophil infiltration into the lungs.

It has become clear that IL-33 has an essential role in various pathophysiologic settings. IL-33 is also involved in the inflammatory tumor microenvironment and the progression of colorectal cancer.[42] IL-33 and ST2 mediate activation of thermogenesis by inducing expression of uncoupling protein 1 in adipocytes, especially for protecting neonates from a cold environment right after birth.[43] IL-33 has another protective role related to the perinatal period. The cytokine induces B cells in the decidual membrane of the placenta to express progesterone-induced blocking factor 1, which has a protective role against preterm labor characterized by T_H1-mediated inflammation.[44] On stimulation with IL-33, ILC2s secrete type 2 cytokines, which also create a beneficial anti-inflammatory environment in the visceral adipose tissue.[45] A high-fat diet exacerbates allergic airway inflammation characterized by infiltration of both ILC2s and ILC3s in the lung. Although the role of IL-33 was unclear in this experimental setting, high IL-33 levels and decreased ILC markers were found in visceral adipose tissue.[2] Indeed, IL-33 has been reported to induce tissue-resident regulatory T cells in some experimental settings.[45]

Several clinical trials with anti–IL-33 antibodies have been planned for allergic diseases, such as asthma, which are recognized to be highly heterogeneous depending on endotypes, such as obesity-associated asthma.[46,47] Therefore it will be important to understand the precise mechanisms and involvement of IL-33 in each endotype of disease to select appropriate patients (ie, those who have markedly gained visceral fat) and decide the timing of treatment for clinical trials using anti–IL-33 antibodies.

TABLE II. Recent *Journal* advances concerning IL-33

- IL-33 induction by ozone was shown.
- Higher levels of IL-33 are seen in obese mice with asthma.
- IL-33 stimulates ILC2s to secrete IL-4 in a food allergy model.
- DUOX1 has been shown to be critical for IL-33 release from cultured human or mouse epithelial cells.
- IL-33 has been shown to activate T_H2 cells with resultant increased production of IL-5 and IL-13.
- Platelets have been shown to express functional IL-33 protein.
- Biomechanical stress has been shown to translocate IL-33 to membrane-bound, secretory-passive, time-dependent release of IL-33 from living cells.
- IL-33 has been shown to be involved in the inflammatory tumor microenvironment and the progression of colorectal cancer.
- IL-33 and ST2 have been shown to mediate activation of thermogenesis by inducing expression of uncoupling protein 1 in adipocytes.

Recent advances published about IL-33 in the *Journal* in 2016 are summarized in Table II.

BARRIER FUNCTION AND MICROBIOME

It became clearer in 2016 that disrupting the epithelial or epidermal barrier triggers inflammation and often forms type 2 innate and acquired immunity against allergens that penetrate these compromised barriers. A number of research studies reported association between the microbiota of these barrier tissues and the onset of allergic disease. Regarding airway epithelium, patients with house dust mite–induced allergic rhinitis had impaired barrier function with decreased expression of tight junction proteins (ie, occludin and zonula occludens 1).[48-53] Regarding the development of asthma, several reports confirmed a positive association with disordered microbiota, although most of them are still proof-of-concept studies. The mechanistic explanations for these associations remain largely unknown but are active areas of investigation. For example, in a population- and national registry–based cohort study including all children born in Denmark, the effect of elective cesarean section was higher than the effect of emergency cesarean section on the risk of asthma,[54] and the effect of cesarean section was associated with early colonization patterns of the neonatal gut but not of the airways in comparison with the effect of natural delivery.[55] Regarding the effect of food supplementation on asthma development, fish-derived n-3 long-chain polyunsaturated fatty acids during pregnancy reduced the risk of persistent wheeze, asthma, and infections of the lower respiratory tract in offspring but not the risk of allergic sensitization.[56] Future studies regarding mechanisms involved in the effect of the microbiome on asthma onset will require a clearer definition of the asthma endotype.

Unlike other allergic diseases, the prevalence of food allergies is rapidly increasing in the 21st century. Growing evidence supports cutaneous sensitization to food antigens as one possible mechanism leading to the failure to develop or to the loss of oral tolerance.[57,58] In this respect, several studies regarding skin barrier function or the skin microbiome have been published in 2016 in relation to atopic dermatitis and other allergic diseases. These studies described associations between neonatal skin barrier dysfunction and the increased risk of food allergy at 2 years of age,[59] between domestic water hardness and chlorine and the increased risk of atopic dermatitis risk,[60] and between early colonization with commensal staphylococci at 2 months of age and a lower risk of atopic dermatitis at 1 year of age.[61]

Concerning the mechanisms involved in skin barrier dysfunction, it was revealed that type 2 and type 17 inflammatory responses develop simultaneously and are counterregulated and driven by TSLP and IL-23, respectively.[62] Although petrolatum is widely used as a moisturizer and is believed to work only as a physical barrier, a recent study revealed that application of petrolatum induced expression of antimicrobial peptides, such as S100A8 and human β-defensin 2; innate immune genes, such as *IL6*; and barrier differentiation markers, such as filaggrin.[62] Regarding the effect of the microbiome on development of allergic diseases, it was shown that the intestinal microbiota of infants allergic to cow's milk is more diverse than that of healthy age-matched control subjects[63] and that marked reductions in skin microbial diversity occur during the exacerbation stage of atopic dermatitis.[64] Extensive studies are needed about the mechanisms involved in these responses, as well as therapeutic approaches based on restoring barrier function.[64,65]

EOSINOPHILIA

Eosinophils were an intense focus over the past year (Fig 2), undoubtedly related to entrance of the first class of anti-eosinophil therapeutics (eg, the anti–IL-5 based antibodies reslizumab and mepolizumab) into routine clinical use for eosinophilic asthma.[66] In addition, the eosinophil-depleting humanized antibody anti–IL-5Ra (benralizumab) was shown to be effective for eosinophilic asthma.[67,68] Eosinophilic gastrointestinal diseases were also a focus area over the past year. Evidence was presented that proton pump inhibitor (PPI)–responsive esophageal eosinophilia (REE) can also respond to dietary therapy,[69] which is consistent with recent reports that PPI-REE has a nearly identical molecular signature to that of eosinophilic esophagitis (EoE), including the cardinal features of allergic inflammation.[70] Further support of the overlap between PPI-REE and EoE was the finding that EoE responds to either diet, steroid, or PPI therapy.[71] Interestingly, PPI treatment was shown to inhibit release of eotaxin-3 (CCL26) by epithelial cells, and this is likely an operational mechanism in patients with EoE and other eosinophilic disorders, such as chronic rhinosinusitis with polyps.[72] Practical guidelines were provided for avoidance of wheat in patients with EoE, with an emphasis that current data do not support extending the wheat elimination to include gluten avoidance.[73] Although levels of food-specific IgE are not increased impressively in patients with EoE, Erwin et al[74] reported that levels of anti-milk IgE correlated with lack of response to milk elimination diets. They reported increased IgG_4/IgE ratios and suggest that this ratio is similar to that seen in patients with tolerance (eg, beekeepers, those living with cats, and those after peanut oral immunotherapy). Notably, Wright et al[75] found increased food-specific IgG_4 levels in the blood and esophagi of patients with EoE. The fibrostenotic complications of EoE are well recognized, but the mechanism has been elusive. In a recent report, cultured longitudinal smooth muscle cells from the esophagus were shown to have a profibrotic response to a rigid substrate. In fact, a rigid cell substrate was shown to induce morphologic and transcriptional changes consistent with EoE.[76] This finding challenges the paradigm of EoE pathogenesis as almost exclusively inflammation dependent

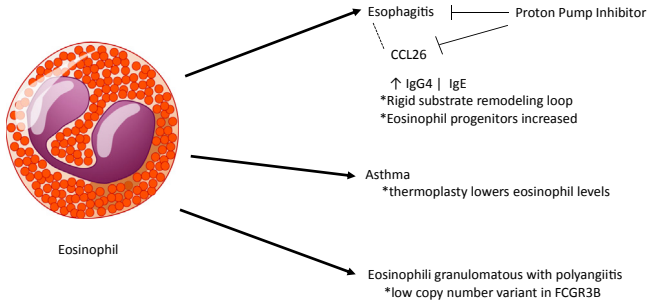


FIG 2. Recent advances about eosinophils.

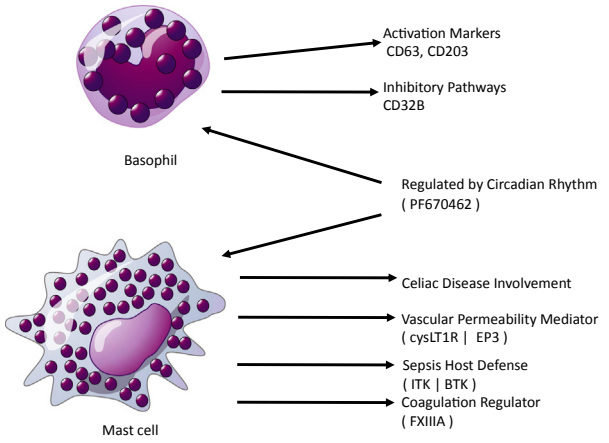


FIG 3. Recent advances about basophils and mast cells.

because it shows that esophageal rigidity has the capacity to cause a positive feedback loop for fibrosis. Eosinophil-associated tissue remodeling is likely to be mediated, as least in part, by periostin-induced eosinophil activation, including TGF-β production, which was demonstrated to be αMβ2 integrin dependent.[77] Although focused on a different disease, another study showed that bronchial thermoplasty in patients with severe asthma decreased blood eosinophil levels. It is interesting to speculate that a reduction in smooth muscle mass by using thermoplasty can disrupt a positive feedback loop, curtailing eosinophilia.[78] Investigators examined circulating biomarkers in patients with EoE and found evidence for increased levels of eosinophil progenitor cells and their correlation with esophageal eosinophil levels.[79] In addition, investigators examined biomarkers in patients with infantile eosinophilic gastroenteritis and found increased levels of TSLP and IL-33 proteins but not a series of other relevant T_H2 markers, calling attention to the potential role of these molecules not only in patients with EoE but also in patients with other eosinophil-associated gastrointestinal disorders.[80]

EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS

The genetic basis of eosinophilic granulomatosis with polyangiitis (EGPA; formerly Churg-Strauss syndrome) was investigated, focusing on copy number variations involving Fcγ receptor (FcγR) genes because low copy number variations have been associated with other autoimmune diseases.[81] Fc fragment of IgG receptor 3B *(FCGR3B)* deficiency (ie, low copy number) was shown to be overrepresented in patients with EGPA, suggesting that this can affect immunoglobulin clearance or perturb the balance between inhibitory and activation Fc receptors, especially because EGPA is characterized by increased autoantibody and IgG_4 levels.[81]

BASOPHILS

A series of important findings concerning basophils were reported in 2016 (Fig 3). Oliver et al[82] reported dysregulated expression of CRTH2 on blood basophils in patients with chronic spontaneous urticaria, suggesting involvement of this receptor as a diagnostic and therapeutic target. Basophils were implicated in allergen-induced bronchoconstriction in patients with mild allergic asthma on the basis of basophil expression of activation markers in the circulation.[83] In an interesting study, both basophils and mast cells were shown to contribute to allergic responses through a circadian clock, demonstrating a marked day/night variation in FcεRI-mediated allergic reactions. PF670462, a selective inhibitor of the key clock component casein kinase 1δ/ε, suppressed IgE-mediated allergic reactions in mouse bone marrow–derived mast cells and basophils and suppressed passive cutaneous anaphylactic reactions in mice. Thus pharmacologically resetting the molecular clock in basophils might be a new strategy for controlling IgE-mediated allergic diseases.[84]

Basophil activation and/or inhibition by IgG subclasses was clarified. Basophils were shown to express CD32a (FcγRIIa), an activation receptor, and CD32b (FcγRIIb), an inhibitory receptor, and IgG_2 and IgG_3 were shown to engage CD32b, with IgG_3 being more potent and IgG_1 and IgG_4 not being able to engage CD32b. These findings draw attention to IgG_3-mediated inhibition through CD32b and direct antigen blockade by IgG_4 when assessing allergic responses before and after immunotherapy.[85]

The Fcγ chain was also shown to be required for basophil IL-4 production.[86] By using mass cytometric profiling of circulating leukocytes in patients with peanut allergy, basophils were shown to be the most responsive cells in the blood to peanut stimulation. Peanut exposure upregulated CD63, a conventional marker of basophil degranulation; modulated FcεRI, CD23, CD16, and CD32; and downregulated expression of CD25 and CD38.[87]

The basophil activation test, which is based on measuring increased levels of CD203c after allergen exposure, is useful in assessing allergen sensitization. A study reported that the additional determination of CD63 combined with CD203c was not superior to CD203c alone in identifying allergic bronchopulmonary aspergillosis in patients with cystic fibrosis.[88]

Basophils have been implicated in mediating a number of allergic responses, including those mediated by TSLP, such as in murine models of atopic dermatitis. However, in a recent report basophil recruitment into the skin was shown to be independent of TSLP receptor expression by basophils, and surprisingly, basophils were shown to ameliorate the atopic dermatitis response. The authors propose that the IL-4– and TSLP-dependent proinflammatory responses were directly mediated by dendritic cells rather than basophils.[89]

MAST CELLS

A number of advances concerning mast cells were reported (Fig 3). In particular, a unique LTD_4-PGE_2 interaction affecting

mast cells through the cysteinyl leukotriene receptor 1 and E-prostanoid receptor 3 was shown to contribute to vascular inflammation in mice. These findings suggest an advantage of dual targeting the cysteinyl leukotriene receptor 1 and E-prostanoid receptor 3 in attenuating inflammation.[23] Mast cells have been shown to be essential to control septic shock in mice. Investigators reported that the nonreceptor tyrosine kinases IL-2–inducible T-cell kinase and Bruton tyrosine kinase negatively regulate mast cell proinflammatory responses to LPS. They reported that the regulatory function downstream of the LPS receptor (Toll-like receptor 4) in mast cells signals through activation of canonical nuclear factor κB, phosphatidylinositol-4,5-bisphosphate 3-kinase/AKT, and p38 signaling pathways.[90] In another report, IL-6 levels were shown to increase human mast cell proliferation and generate an activated phenotype enabled by suppressing proteolytic cleavage of soluble IL-6 receptor from IL-6 receptor and downregulation of a suppressor of cytokine signaling 3 autoinhibitory pathway.[91]

A recent review article focused on the recently identified Mas-related G protein–coupled receptor X2 (MRGPRX2; formerly known as MRGX2), which was shown to be expressed by mast cells and involved in non–IgE-mediated allergic responses to peptidergic drugs.[92] In this review, the possible roles of MRGPRX2 in a variety of responses, including pain, itch, urticaria, asthma, and drug reactions, was reviewed, and potential ways of inhibiting MRGPRX2 were highlighted.

Most studies on mast cell activation have focused on acute activation, such as after 1 dose of anti-IgE, but allergic responses likely involve repetitive allergen exposure. In a recent study, repeated FcεRI-mediated activation of mast cells was assessed by using RNA sequencing. The authors identified 289 genes that were significantly upregulated after repeated anti-IgE, and most of these genes were not found after a single anti-IgE stimulus. Interestingly, the identified genes overlapped with gene expression in tissues with chronic rhinosinusitis, providing new insight into likely operational mechanisms of allergic disease.[93]

A novel mechanism by which the skin microbiota signals the recruitment and maturation of mast cells within the skin through stem cell factor production by using lipoteichoic acid–stimulated keratinocytes was reported.[94] A new disease association for mast cells was reported. In particular, infiltrating mast cells were associated with celiac disease and correlated with the severity of mucosal damage. Interestingly, mast cells were found to release proinflammatory mediators in direct response to the nonimmunodominant fragments of gliadin.[95] Mass spectrometry of mast cell–released proteins was performed. One of the most abundant proteins found was the transglutaminase coagulation Factor XIIIA, and studies in mast cell–deficient mice substantiated a role for mast cells, especially chymase, in regulating transglutaminase coagulation Factor XIIIA plasma activity levels and bleeding times.[96]

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