Advances in allergy, asthma, and immunology

Advances in mechanisms of allergic disease in 2016

Marc E. Rothenberg, MD, PhD,[a] Hirohisa Saito, MD, PhD,[b] and R. Stokes Peebles, Jr, MD[c] *Cincinnati, Ohio, Tokyo, Japan, and Nashville, Tenn*

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

From [a]the Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center; [b]the National Research Institute for Child Health & Development, Tokyo, Japan; and [c]the Department of Medicine, Vanderbilt University Medical Center, Nashville.

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.

Corresponding author: Marc E. Rothenberg, MD, PhD, Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, MLC7028, University of Cincinnati, Cincinnati, OH 45229-3039. E-mail: Rothenberg@cchmc.org.

0091-6749/$36.00

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