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Invited review article

Proallergic cytokines and group 2 innate lymphoid cells in allergic nasal diseases

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Abbreviations:
AFRS, allergic fungal rhinosinusitis; AR, allergic rhinitis; CRS, chronic rhinosinusitis; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; DC, dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; HDM, house dust mite; IL, interleukin; ILC2, group 2 innate lymphoid cell; NAR, non-allergic rhinitis; TLR, toll-like receptor; TSLP, thymic stromal lymphopoietin; OVA, ovalbumin; PBMC, peripheral blood mononuclear cell

ABSTRACT

Recent advances in our understanding of proallergic cytokines and group 2 innate lymphoid cells (ILC2s) indicate their critical roles in type 2 immunity-mediated disorders. Proallergic cytokines, interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin, are released from epithelial cells in inflamed tissues and drive type 2 inflammation by acting on innate and acquired immune systems. ILC2s are an innate immune population that responds to proallergic cytokines by producing type 2 cytokines. In line with allergic disorders in the lung, skin, and intestine, emerging evidence suggests the involvement of proallergic cytokines and ILC2s in allergic nasal diseases such as chronic rhinosinusitis with polyps (CRSwNP), allergic fungal rhinosinusitis, and allergic rhinitis (AR). In CRSwNP patients, both proallergic cytokine levels and ILC2s frequency are increased in the nasal mucosa. Increased proallergic cytokine levels correlate with poorer disease outcomes in CRSwNP. Levels of nasal proallergic cytokines are also elevated in AR patients. In addition, animal studies demonstrate that cytokines are essential for the development of AR. It is becoming clear that the proallergic cytokine/ILC2s axis participates in allergic diseases by multiple mechanisms dependent upon the inflammatory context. Thus, a thorough understanding of these cytokines and ILC2s including their tissue- and disease-specific roles is essential for targeting the pathways to achieve therapeutic applications.

Introduction

Recent major steps forward in our understanding of type 2 immune responses must include the discovery of three proallergic cytokines, interleukin (IL)-25 (also known as IL-17E), IL-33 (also known as IL-1F11), and thymic stromal lymphopoietin (TSLP), as well as a potent innate type 2 cytokine producer, group 2 innate lymphoid cells (ILC2s). IL-25, IL-33, and TSLP are produced by epithelial cells in barrier tissues as well as several immune cells in response to allergen exposure.[1] These cytokines both activate dendritic cells (DCs) to initiate Th2 responses and stimulate Th2 cells to enhance Th2 immunity.[1] In addition to inducing Th2 acquired immune responses, they also stimulate innate type 2 cells including basophils, mast cells, eosinophils, and ILC2s.[1] Thus, they play pivotal roles in both “acquired-type allergy” mediated by activated Th2 cells and antigen-specific IgE, and “innate-type allergy” induced without activation of the acquired immune system.[1,2]

ILC2s are a heterogeneous population comprising natural helper cells,[3] nuocytes,[4] and innate helper type 2 cells,[5] all characterized by a lack of surface lineage markers and the ability to produce large amounts of type 2 cytokines.[6] IL-25, IL-33, and TSLP strongly enhance ILC2 growth and induce the production of IL-5 and IL-13.[6]

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Some studies have shown that ILC2s also produce IL-4, IL-9, granulocyte-macrophage colony-stimulating factor (GM-CSF), and amphiregulin.[7–10] These cytokines promote eosinophilia, mucus production, M2 macrophage development, and tissue repair; thus, ILC2s are important effector cells in type 2 immune responses.[6] In addition to these effector functions, a growing body of evidence indicates that ILC2s mediate Th2 development by promoting DC activation[11,12] or by directly interacting with $CD4^+$ T cells.[8,13]

These observations highlight the critical and complex involvement of the IL-25-, IL-33-, and TSLP-ILC2s axes in the development and exacerbation of allergic inflammation. Thus, it is no surprise that these pathways also have important roles in allergic disorders of the upper airways. Although little information is available compared with other organs such as the lung, skin, or intestine, here we summarize the current understanding of the roles of epithelial-derived proallergic cytokines and ILC2s in nasal allergic diseases, namely chronic rhinosinusitis (CRS) and allergic rhinitis (AR).

Chronic rhinosinusitis

CRS is a nasal inflammatory disease with characteristic symptoms including nasal discharge, nasal congestion, facial pain, and hyposmia or anosmia for 12 weeks or more.[14] CRS can be further divided into two major subtypes, CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP), characterized by the presence or absence of polyps.[14] CRSwNP often accompanies tissue eosinophilia and is considered a Th2-dominated disease, while the immunological phenotype is likely to be skewed towards Th1 in CRSsNP patients.[14] Although CRS is a complex disease and the Th1 vs Th2 classification of the CRS subtypes might be an oversimplification,[15] emerging evidence supports the significant involvement of epithelial-derived proallergic cytokines and ILC2s in CRSwNP.

Human ILC2s were first isolated from lungs, gut, and inflamed nasal polyps,[16] and increased numbers of ILC2s were observed in the polyps from CRSwNP patients compared with nasal mucosa from CRSsNP patients or healthy controls.[7,16–20] Increased ILC2s frequencies in sinus mucosa were correlated with worsening nasal symptoms in CRS patients.[20] In addition, the ILC2 population is concentrated in polyps compared with sinus mucosa of CRSwNP patients.[21] These nasal polyp-derived ILC2s respond to IL-25, IL-33, and TSLP by producing IL-4, IL-5, IL-9, IL-13 and GM-CSF.[7,16,17] Thus, ILC2s may be an important source for type 2 cytokines in nasal polyps and contribute to tissue eosinophilia (Fig. 1). Furthermore, the preferential location of ILC2s in nasal polyps,[21] and gene expression analysis demonstrating that ILC2s express several factors involved in tissue remodeling,[22] suggest these cells contribute to polyp generation in CRSwNP (Fig. 1).

mRNA levels of IL-33 receptor, *ST2* (also known as *IL1RL1*), and numbers of $ST2^+$ cells are increased in polyps from CRSwNP or eosinophilic CRS patients.[17,23,24] Furthermore, an association between *ST2* polymorphisms and CRS was reported.[25] Among $CD45^+$ cells in the nasal mucosa from CRSwNP patients, ILC2s were the dominant source of IL-13 by *in vitro* stimulation of cells with IL-2 plus IL-33.[17] Although some studies have demonstrated that *IL33* expression levels were not altered among CRSwNP, CRSsNP, and healthy controls,[17,18,23] an interesting observation indicated that nasal epithelial cells from treatment-resistant CRSwNP patients expressed higher levels of *IL33* compared with treatment-responsive CRSwNP patients.[26] Therefore, altered IL-33 expression or an enhanced response of ILC2s to this cytokine in CRSwNP patients may aggravate disease severity and compromise the efficacy of treatments.

The expression levels of *TSLP* and *TSLPR* (also known as *CRLF2*) were higher in the nasal mucosa from CRSwNP[7,27,28] or eosinophilic CRS[29] patients compared with CRSsNP or healthy controls. In addition, nasal epithelial cells from CRSwNP patients had a greater up-regulation of *TSLP* expression in response to stimulation by poly I:C, a Toll-like receptor (TLR)3 ligand, compared with healthy controls.[7] Importantly, *TSLP* expression levels in nasal mucosa correlated to the polyp scores in CRS patients.[29] In addition to the observation of increased *TSLP* mRNA transcription, TSLP protein undergoes proteolytic cleavage by proteases in nasal polyps which enhances its bioactivity.[30] Increased TSLP activity in CRSwNP nasal mucosa may contribute to increased activation of DCs,[28] mast cells,[30] and ILC2s[7,16] (Fig. 1). ILC2s from nasal polyps also responded to IL-25,[16] and a study demonstrated elevated *IL25* levels in CRSwNP patients (Fig. 1).[31] Although the study did not observe an association between disease severity and *IL33* or *TSLP* levels, they showed that increased *IL25* levels correlated with poorer computed tomography (CT) scores in CRSwNP.[31]

These observations suggest the critical involvement of proallergic cytokines/ILC2s axis in the pathogenesis of CRSwNP. Although the relative contribution of ILC2s and Th2 cells in the type 2 milieu in CRSwNP is currently not clear, a study demonstrated that the number of ILC2s and Th2 cells was significantly correlated in nasal mucosa from CRSwNP patients.[18] Thus, ILC2s and Th2 cells may be simultaneously activated in the local allergic environment as both populations can respond to epithelial-derived proallergic cytokines.[1] Alternatively, they might cross-regulate each other as the dialogue between ILC2s and $CD4^+$ T cells was demonstrated in lungs[8] and intestines (Fig. 1).[13]

Allergic fungal rhinosinusitis (AFRS) is an endotype of CRS caused by bacterial or fungal infection,[14,32] and characterized by the presence of fungal hyphae in mucin in the sinus cavity, and serum fungi-specific IgE.[14,32] The local elevation of type 2 cytokines, IL-4, IL-5, and IL-13, is often observed in AFRS; thus the disease is considered a Th2-dominant type of CRS.[14,32] Currently, no study has directly investigated the role of proallergic cytokines in the pathogenesis of AFRS.[32] However, because both AFRS and CRSwNP are linked to type 2 immunity,[14,32] and fungi extracts or TLR ligands (fungal or bacterial accumulation present in AFRS patients) induce the expression of *IL25*,[33] *IL33*,[17] and *TSLP*[7] in nasal epithelial cells, epithelial-derived proallergic cytokines and ILC2s may also play important roles in AFRS analogous to that in CRSwNP. Further study is required to fully understand the subtype specific roles of proallergic cytokines and ILC2s in CRS.

Allergic rhinitis

AR is an IgE-mediated type 1 hypersensitivity reaction in nasal mucosa caused by nasal allergen exposure.[34,35] The characteristic symptoms of AR are sneezing, rhinorrhea, and nasal congestion caused by IgE-mast-cell-mediated early-phase responses of AR (5–30 min after allergen exposure).[34,35] In addition, type 2 cytokines and chemokines mediate the cellular influx into nasal mucosa, typically eosinophilia, which cause the late-phase responses of AR (approximately 6–24 h after allergen exposure) resulting in tissue damage and remodeling.[34,35] Because both AR and CRS are nasal inflammatory diseases, they are associated with each other with an estimated prevalence of AR of 60% in CRS patients.[14]

Several lines of evidence have indicated increased epithelial proallergic cytokine levels in AR patients. Three cytokines, IL-25,[36] IL-33,[37,38] and TSLP[36] were detected in the nasal lavage from patients with house dust mite (HDM)[36,37] or Japanese cedar pollen[38] AR. *IL33*,[39,40] *ST2*,[40] and *TSLP*[27,41–43] mRNA levels were high in nasal epithelia from several types of AR patients. $CD4^+$ cells in peripheral blood mononuclear cells (PBMCs) from AR patients with birch, grass,[44] or Japanese cedar[45] pollen sensitization expressed high levels of *IL25R* (also known as *IL17RB*) mRNA.[44,45]

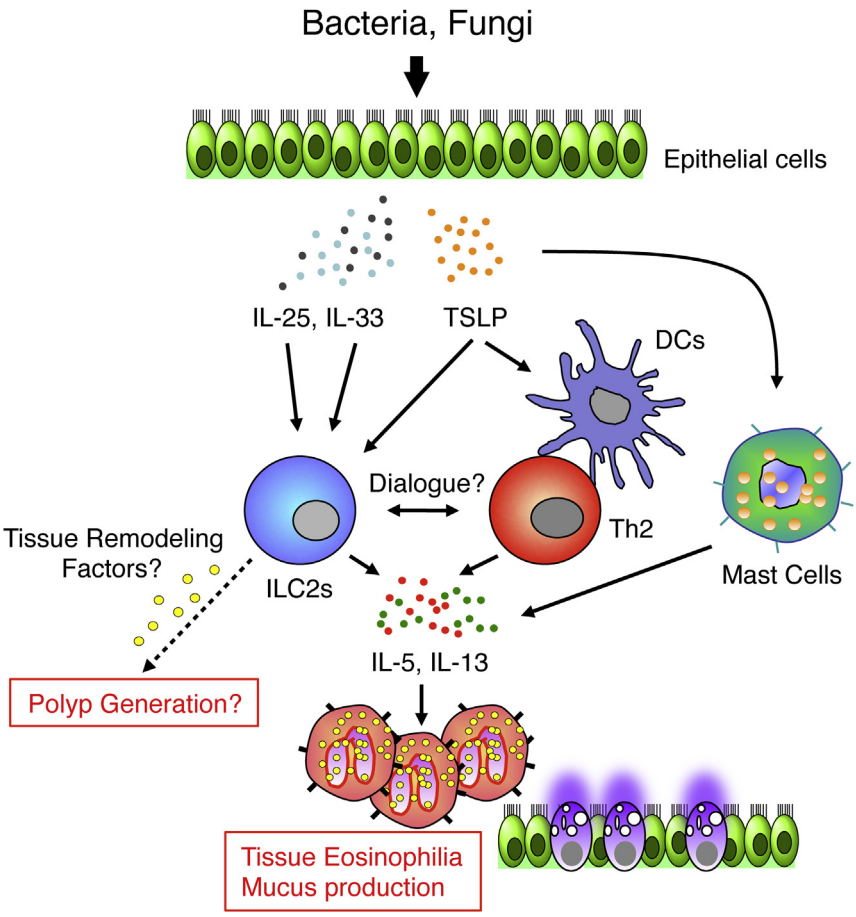

Fig. 1. Proallergic cytokines and group 2 innate lymphoid cells in chronic rhinosinusitis with nasal polyps. Although the cause of chronic rhinosinusitis with nasal polyps (CRSwNP) varies among patients, bacterial or fungal infection induces the production of proallergic cytokines from nasal epithelial cells. IL-25, IL-33, and TSLP stimulate group 2 innate lymphoid cells (ILC2s) to produce type 2 cytokines, IL-5 and IL-13. TSLP activates dendritic cells (DCs) to activate Th2 cells, and contributes to IL-5 production from mast cells. Because the numbers of ILC2s and Th2 cells are associated in the nasal sinus, these cells might cross-regulate each other. IL-5 and IL-13 induce eosinophilic infiltration into the nasal mucosa. ILC2s might also produce tissue remodeling factors that could be involved in polyp generation during disease.

A genome-wide association study predicted genetic polymorphisms in *IL33* locus linked to Japanese cedar pollenosis,[38] and serum IL-33 levels seemed to correlate with disease severity of grass and/or tree pollen AR.[46] In addition, an animal study using *Il33*$^{-/-}$ mice demonstrated a pivotal role for IL-33 in ragweed-specific AR.[39] After nasal ragweed pollen challenge of ragweed-sensitized mice, *Il33*$^{-/-}$ mice showed attenuated sneezing and eosinophilic and basophilic infiltration into the nose with lower serum ragweed-specific IgE titers and reduced ragweed-specific Th2 cell responses compared with wild-type controls.[39] IL-33 augments IgE-mediated histamine release from mast cells and IgE-independent cytokine and chemokine production from mast cells and basophils (Fig. 2).[39] In addition to gene-targeted mouse experiments, the treatment of ovalbumin (OVA)-sensitized mice with anti-IL-33 antibodies at the nasal OVA-challenge phase attenuated nose scratching behavior and nasal cellular infiltration[47] in mice, indicating the therapeutic potential of targeting IL-33 in post-sensitized AR patients.

As for IL-33, polymorphisms in *TSLP* locus are also associated with AR,[48–50] and nasal TSLP expression levels are linked to disease severity.[42,43] As TSLP is regarded as the master regulator for Th2 development by activating DCs,[1] TSLP-responsive CD1c^{+} DCs are recruited to nasal mucosa of grass pollen AR patients after allergen challenge.[51] TSLP-activated CD1c^{+} DCs strongly induce the proliferation of, and type 2 cytokine production from allergen-specific Th2 cells (Fig. 2).[51] An animal study using OVA-sensitized mice demonstrated that anti-TSLP antibody treatment at the nasal OVA-challenge phase ameliorated scratching behavior and nasal goblet cell hyperplasia.[52] Thus, analogous to the role of TSLP in asthma models, TSLP may have a critical role in developing Th2 responses in nasal mucosa (Fig. 2). Although the relative contribution of IL-33 and TSLP in AR is not clear, a study showed a positive correlation between IL-33 and TSLP levels in nasal mucosa,[18] suggesting that these cytokines cross-regulate each other either directly or indirectly.

IL-25 is also detected in nasal lavage from HDM AR patients,[36] and a mouse model of OVA-specific AR demonstrated up-regulated *Il25* mRNA levels in diseased nasal mucosa.[53] Allergen stimulation of basophils from birch or grass pollen AR patients, but not from healthy controls, up-regulated IL-25R expression on PBMCs.[44] The same study demonstrated that IL-25 stimulation of basophils inhibited cellular apoptosis and enhanced IgE-induced degranulation (Fig. 2).[44] In addition, IL-25 enhanced poly I:C-induced TSLP production from primary human nasal epithelial cells.[36]

Despite our understanding of the pivotal roles of epithelial-derived proallergic cytokines in AR pathogenesis, the role of ILC2s in AR is poorly understood. AR is an IgE- and Th2-dependent disorder[34,35] and the development of AR was completely abrogated in *Rag2*$^{-/-}$ mice, which lack both T and B cells but retain ILC2s, when

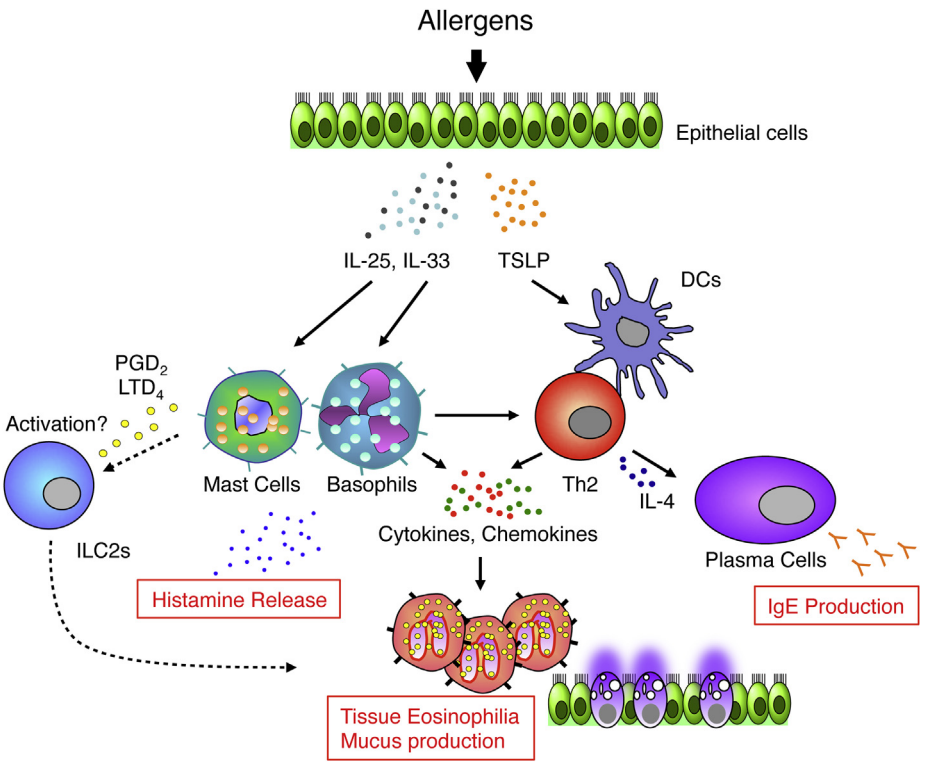

Fig. 2. Proallergic cytokines and group 2 innate lymphoid cells in allergic rhinitis. Nasal epithelial cells stimulated with allergens produce proallergic cytokines. TSLP activates dendritic cells (DCs) to drive allergen-specific Th2 cells. Following IgE crosslinking, IL-33 augments mast cell degranulation and IL-25 augments basophil degranulation, which enhances the early phase response of allergic rhinitis (AR). IL-33 also induces cytokines and chemokines production from mast cells and basophils, resulting in enhanced local type 2 immune responses. Allergen stimulation of mast cells and basophils induces the rapid release of lipid mediators, such as prostaglandin D_2 (PGD2) or leukotriene D_4 (LTD4), which may cause the temporal activation of group 2 innate lymphoid cells (ILC2s). Generation of nasal type 2 milieu results in eosinophilic infiltration and mucus production that mediates the late phase responses of AR.

mice were nasally sensitized with ragweed pollen.[54] This indicated that ILC2s alone cannot mediate the symptoms of AR including tissue eosinophilia,[54] which can be induced solely by activating ILC2s in lungs.[55] ILC2s are present in mouse nasal mucosa in the steady state,[54] although ILC2s do not expand in the allergic nasal mucosa (Y.K., S.A., and T.Y. unpublished observation). In a human study, the numbers and cytokine-producing activities of ILC2s in PBMCs from AR patients were comparable to those from healthy controls, whereas allergic asthma patients showed an increased number of ILC2s and increased cytokine production from ILC2s in PBMCs.[56] However, another human study of cat allergen-sensitized AR patients demonstrated an increased ILC2 frequency in PBMCs, 4 h after nasal cat allergen exposure.[57] Thus, even though AR conditions do not affect the state of ILC2s in the nose or periphery, these cells could be temporarily activated after allergen exposure and participate in the production of type 2 cytokines collaborating with other cells, such as Th2 cells, basophils, and mast cells. In addition to proallergic cytokines, ILC2s were activated by prostaglandin D_2[58] or leukotriene D_4[59] rapidly released from basophils and mast cells by allergen exposure in AR (Fig. 2).[34] Because recent studies have demonstrated the involvement of ILC2s in the development of Th2-acquired immunity in lungs,[8,11,12] whether this is also the case in nasal allergy should be investigated. The exact contribution of ILC2s in AR might be determined by using recently established mouse models in which the ILC2 population is specifically abrogated.[11,60]

AR is diagnosed by detecting systemic or local allergen-specific IgE in individuals who have rhinitis symptoms.[34,35] Rhinitis patients with negative allergen-specific IgE are classified as non-allergic rhinitis (NAR).[61] NAR is a heterogeneous population and some NAR patients show nasal eosinophilia and/or mastocytosis.[62,63] Importantly, NAR patients with eosinophils and/or mast cells have a lower quality of life and a higher risk for comorbidities compared with other NAR patients.[62,63] Because the presence of eosinophils or mast cells is reminiscent of type 2 inflammation, it is of interest to investigate the involvement of proallergic cytokines and ILC2s in NAR with eosinophils and/or mast cells. These pathways might activate type 2 inflammation without the presence of specific Th2-acquired immune responses.

Conclusions

Accumulating evidence indicates that proallergic cytokines and ILC2s pathways contribute to the pathogenesis of allergic nasal diseases. Importantly, some studies have shown that proallergic cytokine levels[29,31,42,43,46] or ILC2 numbers[20] correlated to the severity of nasal allergic diseases, implicating the control of these factors might be beneficial for the treatment of the diseases. In addition, the proallergic cytokine/ILC2s axis might mediate treatment resistivity in some allergy conditions.[26,64] Thus, targeting proallergic cytokines or ILC2s may improve treatment efficacy, especially in treatment-resistant patients. In addition to using cytokine-neutralizing antibodies, recent studies have demonstrated the potential therapeutic use of small molecule inhibitors against the proallergic cytokine/ILC2 pathway. For example, a STAT5 inhibitor, Pimozide, blocked the TSLP/STAT5 pathway and improved corticosteroid resistance of ILC2s in a lung inflammation model.[64] An anti-inflammatory lipid, lipoxin A_4, inhibited ILC2s activation.[65] Furthermore, ILC2-activating lipid mediators, prostaglandin D_2 or leukotriene D_4, pathways can be inhibited by

currently available anti-allergy medicines.[66] However, despite the rapid advances in our understanding of the basic biology of proallergic cytokines and ILC2s, little is known about their specific roles in nasal tissues. Further studies investigating nasal diseases need to be conducted, and a deeper understanding of proallergic cytokines and ILC2s in the context of specific disease conditions may offer opportunities for the development of novel clinical interventions for nasal allergic diseases.

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Conflict of interest

The authors have no conflict of interest to declare.

References

1. Licona-Limon P, Kim LK, Palm NW, Flavell RA. TH2, allergy and group 2 innate lymphoid cells. *Nat Immunol* 2013;**14**:536–42.
2. Yoshimoto T, Matsushita K. Innate-type and acquired-type allergy regulated by IL-33. *Allergol Int* 2014;**63**(Suppl. 1):3–11.
3. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature* 2010;**463**:540–4.
4. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 2010;**464**:1367–70.
5. Price AE, Liang HE, Sullivan BM, Reinhardt RL, Eisley CJ, Erle DJ, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci U S A* 2010;**107**:11489–94.
6. McKenzie AN, Spits H, Eberl G. Innate lymphoid cells in inflammation and immunity. *Immunity* 2014;**41**:366–74.
7. Mjosberg J, Bernink J, Golebski K, Karrich JJ, Peters CP, Blom B, et al. The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. *Immunity* 2012;**37**:649–59.
8. Drake LY, Iijima K, Kita H. Group 2 innate lymphoid cells and CD4(+) T cells cooperate to mediate type 2 immune response in mice. *Allergy* 2014;**69**:1300–7.
9. Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-Owsiak D, Wang X, et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med* 2013;**210**:2939–50.
10. Turner JE, Morrison PJ, Wilhelm C, Wilson M, Ahlfors H, Renauld JC, et al. IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. *J Exp Med* 2013;**210**:2951–65.
11. Halim TY, Steer CA, Matha L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity* 2014;**40**:425–35.
12. Gold MJ, Antignano F, Halim TY, Hirota JA, Blanchet MR, Zaph C, et al. Group 2 innate lymphoid cells facilitate sensitization to local, but not systemic, TH2-inducing allergen exposures. *J Allergy Clin Immunol* 2014;**133**:1142–8.
13. Oliphant CJ, Hwang YY, Walker JA, Salimi M, Wong SH, Brewer JM, et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity* 2014;**41**:283–95.
14. Ocampo CJ, Grammer LC. Chronic rhinosinusitis. *J Allergy Clin Immunol Pract* 2013;**1**:205–11. quiz 12-3.
15. Katotomichelakis M, Tantilipikorn P, Holtappels G, De Ruyck N, Feng L, Van Zele T, et al. Inflammatory patterns in upper airway disease in the same geographical area may change over time. *Am J Rhinol Allergy* 2013;**27**:354–60.
16. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat Immunol* 2011;**12**:1055–62.
17. Shaw JL, Fakhri S, Citardi MJ, Porter PC, Corry DB, Kheradmand F, et al. IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosinusitis with nasal polyps. *Am J Respir Crit Care Med* 2013;**188**:432–9.
18. Miljkovic D, Bassiouni A, Cooksley C, Ou J, Hauben E, Wormald PJ, et al. Association between group 2 innate lymphoid cells enrichment, nasal polyps and allergy in chronic rhinosinusitis. *Allergy* 2014;**69**:1154–61.
19. Walford HH, Lund SJ, Baum RE, White AA, Bergeron CM, Husseman J, et al. Increased ILC2s in the eosinophilic nasal polyp endotype are associated with corticosteroid responsiveness. *Clin Immunol* 2014;**155**:126–35.
20. Ho J, Bailey M, Zaunders J, Mrad N, Sacks R, Sewell W, et al. Group 2 innate lymphoid cells (ILC2s) are increased in chronic rhinosinusitis with nasal polyps or eosinophilia. *Clin Exp Allergy* 2015;**45**:394–403.
21. Ho J, Bailey M, Zaunders J, Mrad N, Sacks R, Sewell W, et al. Cellular comparison of sinus mucosa vs polyp tissue from a single sinus cavity in chronic rhinosinusitis. *Int Forum Allergy Rhinol* 2014;**5**:14–27.
22. Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* 2012;**36**:451–63.
23. Baba S, Kondo K, Kanaya K, Suzukawa K, Ushio M, Urata S, et al. Expression of IL-33 and its receptor ST2 in chronic rhinosinusitis with nasal polyps. *Laryngoscope* 2014;**124**:E115–22.
24. Plager DA, Kahl JC, Asmann YW, Nilson AE, Pallanch JF, Friedman O, et al. Gene transcription changes in asthmatic chronic rhinosinusitis with nasal polyps and comparison to those in atopic dermatitis. *PLoS One* 2010;**5**:e11450.
25. Castano R, Bosse Y, Endam LM, Desrosiers M. Evidence of association of interleukin-1 receptor-like 1 gene polymorphisms with chronic rhinosinusitis. *Am J Rhinol Allergy* 2009;**23**:377–84.
26. Reh DD, Wang Y, Ramanathan Jr M, Lane AP. Treatment-recalcitrant chronic rhinosinusitis with polyps is associated with altered epithelial cell expression of interleukin-33. *Am J Rhinol Allergy* 2010;**24**:105–9.
27. Kimura S, Pawankar R, Mori S, Nonaka M, Masuno S, Yagi T, et al. Increased expression and role of thymic stromal lymphopoietin in nasal polyposis. *Allergy Asthma Immunol Res* 2011;**3**:186–93.
28. Liu T, Li TL, Zhao F, Xie C, Liu AM, Chen X, et al. Role of thymic stromal lymphopoietin in the pathogenesis of nasal polyposis. *Am J Med Sci* 2011;**341**:40–7.
29. Ouyang Y, Fan E, Li Y, Wang X, Zhang L. Clinical characteristics and expression of thymic stromal lymphopoietin in eosinophilic and non-eosinophilic chronic rhinosinusitis. *ORL J Otorhinolaryngol Relat Spec* 2013;**75**:37–45.
30. Nagarkar DR, Poposki JA, Tan BK, Comeau MR, Peters AT, Hulse KE, et al. Thymic stromal lymphopoietin activity is increased in nasal polyps of patients with chronic rhinosinusitis. *J Allergy Clin Immunol* 2013;**132**:593–600. e12.
31. Lam M, Hull L, McLachlan R, Snidvongs K, Chin D, Pratt E, et al. Clinical severity and epithelial endotypes in chronic rhinosinusitis. *Int Forum Allergy Rhinol* 2013;**3**:121–8.
32. Plonk DP, Luong A. Current understanding of allergic fungal rhinosinusitis and treatment implications. *Curr Opin Otolaryngol Head Neck Surg* 2014;**22**:221–6.
33. Kouzaki H, Tojima I, Kita H, Shimizu T. Transcription of interleukin-25 and extracellular release of the protein is regulated by allergen proteases in airway epithelial cells. *Am J Respir Cell Mol Biol* 2013;**49**:741–50.
34. Borish L. Allergic rhinitis: systemic inflammation and implications for management. *J Allergy Clin Immunol* 2003;**112**:1021–31.
35. Brozek JL, Bousquet J, Baena-Cagnani CE, Bonini S, Canonica GW, Casale TB, et al. Allergic rhinitis and its impact on asthma (ARIA) guidelines: 2010 revision. *J Allergy Clin Immunol* 2010;**126**:466–76.
36. Xu G, Zhang L, Wang DY, Xu R, Liu Z, Han DM, et al. Opposing roles of IL-17A and IL-25 in the regulation of TSLP production in human nasal epithelial cells. *Allergy* 2010;**65**:581–9.
37. Asaka D, Yoshikawa M, Nakayama T, Yoshimura T, Moriyama H, Otori N. Elevated levels of interleukin-33 in the nasal secretions of patients with allergic rhinitis. *Int Arch Allergy Immunol* 2012;**158**(Suppl. 1):47–50.
38. Sakashita M, Yoshimoto T, Hirota T, Harada M, Okubo K, Osawa Y, et al. Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. *Clin Exp Allergy* 2008;**38**:1875–81.
39. Haenuki Y, Matsushita K, Futatsugi-Yumikura S, Ishii KJ, Kawagoe T, Imoto Y, et al. A critical role of IL-33 in experimental allergic rhinitis. *J Allergy Clin Immunol* 2012;**130**:184–94. e11.
40. Kamekura R, Kojima T, Takano K, Go M, Sawada N, Himi T. The role of IL-33 and its receptor ST2 in human nasal epithelium with allergic rhinitis. *Clin Exp Allergy* 2012;**42**:218–28.
41. Kamekura R, Kojima T, Koizumi J, Ogasawara N, Kurose M, Go M, et al. Thymic stromal lymphopoietin enhances tight-junction barrier function of human nasal epithelial cells. *Cell Tissue Res* 2009;**338**:283–93.
42. Zhu DD, Zhu XW, Jiang XD, Dong Z. Thymic stromal lymphopoietin expression is increased in nasal epithelial cells of patients with mugwort pollen sensitive-seasonal allergic rhinitis. *Chin Med J (Engl)* 2009;**122**:2303–7.
43. Mou Z, Xia J, Tan Y, Wang X, Zhang Y, Zhou B, et al. Overexpression of thymic stromal lymphopoietin in allergic rhinitis. *Acta Otolaryngol* 2009;**129**:297–301.
44. Wang H, Mobini R, Fang Y, Barrenas F, Zhang H, Xiang Z, et al. Allergen challenge of peripheral blood mononuclear cells from patients with seasonal allergic rhinitis increases IL-17RB, which regulates basophil apoptosis and degranulation. *Clin Exp Allergy* 2010;**40**:1194–202.
45. Matsumoto Y, Noguchi E, Imoto Y, Nanatsue K, Takeshita K, Shibasaki M, et al. Upregulation of IL17RB during natural allergen exposure in patients with seasonal allergic rhinitis. *Allergol Int* 2011;**60**:87–92.
46. Gluck J, Rymarczyk B, Rogala B. Serum IL-33 but not ST2 level is elevated in intermittent allergic rhinitis and is a marker of the disease severity. *Inflamm Res* 2012;**61**:547–50.
47. Kim YH, Yang TY, Park CS, Ahn SH, Son BK, Kim JH, et al. Anti-IL-33 antibody has a therapeutic effect in a murine model of allergic rhinitis. *Allergy* 2012;**67**:183–90.
48. Ramasamy A, Curjuric I, Coin LJ, Kumar A, McArdle WL, Imboden M, et al. A genome-wide meta-analysis of genetic variants associated with allergic

rhinitis and grass sensitization and their interaction with birth order. *J Allergy Clin Immunol* 2011;**128**:996–1005.
49. Birben E, Sahiner UM, Karaaslan C, Yavuz TS, Cosgun E, Kalayci O, et al. The genetic variants of thymic stromal lymphopoietin protein in children with asthma and allergic rhinitis. *Int Arch Allergy Immunol* 2014;**163**:185–92.
50. Nilsson D, Henmyr V, Hallden C, Sall T, Kull I, Wickman M, et al. Replication of genomewide associations with allergic sensitization and allergic rhinitis. *Allergy* 2014;**69**:1506–14.
51. Melum GR, Farkas L, Scheel C, Van Dieren B, Gran E, Liu YJ, et al. A thymic stromal lymphopoietin-responsive dendritic cell subset mediates allergic responses in the upper airway mucosa. *J Allergy Clin Immunol* 2014;**134**:613–21. e7.
52. Miyata M, Hatsushika K, Ando T, Shimokawa N, Ohnuma Y, Katoh R, et al. Mast cell regulation of epithelial TSLP expression plays an important role in the development of allergic rhinitis. *Eur J Immunol* 2008;**38**:1487–92.
53. Li Z, Wang H, Liu L. Interleukin-25 enhances allergic inflammation through p38MAPK and NF-kappaB pathways in mouse models of allergic rhinitis. *Iran J Allergy Asthma Immunol* 2014;**13**:412–9.
54. Kato Y, Akasaki S, Muto-Haenuki Y, Fujieda S, Matsushita K, Yoshimoto T. Nasal sensitization with ragweed pollen induces local-allergic-rhinitis-like symptoms in mice. *PLoS One* 2014;**9**:e103540.
55. Yasuda K, Muto T, Kawagoe T, Matsumoto M, Sasaki Y, Matsushita K, et al. Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice. *Proc Natl Acad Sci U S A* 2012;**109**:3451–6.
56. Bartemes KR, Kephart GM, Fox SJ, Kita H. Enhanced innate type 2 immune response in peripheral blood from patients with asthma. *J Allergy Clin Immunol* 2014;**134**:671–8. e4.
57. Doherty TA, Scott D, Walford HH, Khorram N, Lund S, Baum R, et al. Allergen challenge in allergic rhinitis rapidly induces increased peripheral blood type 2 innate lymphoid cells that express CD84. *J Allergy Clin Immunol* 2014;**133**:1203–5.
58. Xue L, Salimi M, Panse I, Mjosberg JM, McKenzie AN, Spits H, et al. Prostaglandin D2 activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on TH2 cells. *J Allergy Clin Immunol* 2014;**133**:1184–94.
59. Doherty TA, Khorram N, Lund S, Mehta AK, Croft M, Broide DH. Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production. *J Allergy Clin Immunol* 2013;**132**:205–13.
60. Halim TY, MacLaren A, Romanish MT, Gold MJ, McNagny KM, Takei F. Retinoic-acid-receptor-related orphan nuclear receptor alpha is required for natural helper cell development and allergic inflammation. *Immunity* 2012;**37**:463–74.
61. Incorvaia C, Fuiano N, Canonica GW. Seeking allergy when it hides: which are the best fitting tests? *World Allergy Organ J* 2013;**6**:11.
62. Gelardi M, Maselli Del Giudice A, Fiorella ML, Soleti P, Di Gioacchino M, Conti CM, et al. Quality of life in non-allergic rhinitis depends on the predominant inflammatory cell type. *J Biol Regul Homeost Agents* 2008;**22**:73–81.
63. de Corso E, Battista M, Pandolfini M, Liberati L, Baroni S, Romanello M, et al. Role of inflammation in non-allergic rhinitis. *Rhinology* 2014;**52**:142–9.
64. Kabata H, Moro K, Fukunaga K, Suzuki Y, Miyata J, Masaki K, et al. Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation. *Nat Commun* 2013;**4**:2675.
65. Barnig C, Cernadas M, Dutile S, Liu X, Perrella MA, Kazani S, et al. Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. *Sci Transl Med* 2013;**5**: 174ra26.
66. Harvima IT, Levi-Schaffer F, Draber P, Friedman S, Polakovicova I, Gibbs BF, et al. Molecular targets on mast cells and basophils for novel therapies. *J Allergy Clin Immunol* 2014;**134**:530–44.