Immunological Reviews

Rosemarie H. DeKruyff
Sanhong Yu
Hye Young Kim
Dale T. Umetsu

# Innate immunity in the lung regulates the development of asthma

Authors' addresses
*Rosemarie H. DeKruyff*[1], *Sanhong Yu*[1], *Hye Young Kim*[1], *Dale T. Umetsu*[1,2]
[1]Division of Immunology and Allergy, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA.
[2]Genentech, South San Francisco, CA, USA.

Correspondence to:
*Dale T. Umetsu*
Genentech
One DNA Way, MS 453b
South San Francisco, CA 94080, USA
Tel.: +1 650 283 2135
e-mail: umetsud@gene.com

Acknowledgements
D. T. U. is an employee of Genentech. The authors declare no conflicts of interest.

This article is part of a series of reviews covering Mucosal Immunity appearing in Volume 260 of Immunological Reviews.

*Immunological Reviews 2014*
*Vol. 260: 235–248*

Immunological Reviews
0105-2896

**Summary:** The lung, while functioning as a gas exchange organ, encounters a large array of environmental factors, including particulate matter, toxins, reactive oxygen species, chemicals, allergens, and infectious microbes. To rapidly respond to and counteract these elements, a number of innate immune mechanisms have evolved that can lead to lung inflammation and asthma, which is the focus of this review. These innate mechanisms include a role for two incompletely understood cell types, invariant natural killer T (iNKT) cells and innate lymphoid cells (ILCs), which together produce a wide range of cytokines, including interleukin-4 (IL-4), IL-5, IL-13, interferon-γ, IL-17, and IL-22, independently of adaptive immunity and conventional antigens. The specific roles of iNKT cells and ILCs in immunity are still being defined, but both cell types appear to play important roles in the lungs, particularly in asthma. As we gain a better understanding of these innate cell types, we will acquire great insight into the mechanisms by which allergic and non-allergic asthma phenotypes develop.

Keywords: *airway hyperreactivity, innate lymphoid cells, NKT cells, allergy, influenza, obesity*

## Introduction

The lung, in functioning as a gas exchange organ, requires a very large surface area of approximately 20 $m^2$, which is >60 times the body surface area. This large pulmonary surface interfaces with the environment and endures daily contact with thousands of liters of inhaled air that frequently contains infectious particles, including bacteria, viruses, fungi, particulate matter, combustion exhaust particles, toxins, free radicals, reactive oxygen species, chemicals, and allergens. Because these agents may damage the lung and compromise its critical gas exchange and oxygen delivery function, the lung has evolved a variety of strategies to deal with these encounters, to control infection and inflammation, and maintain homeostasis. These strategies include mechanisms to filter and dispose of particulate matter, maintain the humid environment in the lungs, and rapidly counter infectious microbes or commensals infecting or colonizing the lungs (1).

Immunologically, the focus of past studies of immune responses in the lungs has been on adaptive immunity, particularly on memory responses to infections and allergens. Antigen-specific adaptive $CD4^+$ and $CD8^+$ T cells responding to immunological stimuli in the lung and producing a host of cytokines or expressing cytolytic activity have been studied extensively for decades. In addition, antigen-specific antibody production by B cells plays a critical role in infection control or allergy. These adaptive pulmonary responses can involve innate cells, including several different dendritic cell (DC) subsets, which take up antigen/allergen or infectious particles to sensitize and activate T cells and B cells. In addition, macrophages, neutrophils, eosinophils, mast cells, and basophils, as well as epithelial cells producing a host of chemokines and inflammatory mediators, participate in these adaptive responses by amplifying or providing accessory effector cell activity. Recently however, an important role in the lung for newly described innate cell types, called innate lymphoid cells (ILCs) that can function independently as effector cells, or can function in collaboration with other innate-like cell types such as Natural Killer T (NKT) cells has been described. In this review, we discuss recent studies of innate immunity in the lung involving ILCs and NKT cells, primarily in the context of asthma.

## Heterogeneity in asthma

Asthma has been considered for more than 2 decades to be an immunological disease of the airways mediated by adaptive immunity and Th2 cells. Allergen-specific Th2 cells, described initially almost 30 years ago, are thought to critically orchestrate inflammatory responses in the lung that result in the symptoms of asthma, particularly of allergic asthma, the most common form of asthma. Th2 cells produce IL-4, IL-5, IL-9, and IL-13, which enhance allergen-specific IgE production from B cells, enhance the growth and differentiation of mast cells and eosinophils and the recruitment and activation of basophils, and directly cause airway hyperreactivity (AHR), a cardinal feature of asthma. Although innate cells including DCs, mast cells, basophils, and eosinophils, as well as airway epithelial cells were known for many years to participate in this adaptive immune response in the lungs, more recently it has become clear that asthma involves much more than adaptive immunity and that asthma is a heterogeneous and complex syndrome, with at least several distinct phenotypes, including both allergic and non-allergic forms, involving non-adaptive immunity.

Thus, although 70-80% of patients with asthma are sensitized to environmental allergens (e.g. dust mite, fungi, and pollens), asthma is also often triggered by non-allergic environmental factors, such as air pollution (ozone, diesel particles, cigarette smoke), viral infection, and in association with obesity. While such non-allergic triggers might function to enhance allergic sensitization or to enhance the function of Th2 effector cells, these non-allergic triggers potently cause asthma symptoms in patients regardless of allergic sensitization, and presumably independently of adaptive immunity. The existence non-allergic (innate) pathways leading to asthma in at least in some patients, distinct from allergic adaptive pathways, is also suggested by therapeutic trials with monoclonal antibodies that target a single Th2 cytokine (IL-4, IL-5 or IL-13), each demonstrating the greatest benefit in a subset of asthma patients (2–4). The mechanisms by which these non-allergic agents trigger asthma symptoms have been extremely difficult to study in patients, given that nearly all patients are exposed to most, although not necessarily developing symptoms with all, of these agents.

## Non-Th2 cell models of asthma

To help clarify the mechanisms of asthma, mouse models were introduced beginning several decades ago. The initial models focused on allergic asthma and involved allergen sensitization followed by allergen challenge. Such reductionist models very effectively established an important role for allergen-specific T-helper 2 (Th2) cells and adaptive immunity, as well as basophils, eosinophils, and mast cells in some models, and paved the way for confirmatory humans studies showing a critical role of Th2 cells in asthma (5). However, these models were often considered inadequate in that they could not explain the complex nature of asthma, i.e. asthma triggered by air pollution, viral infection, obesity, or oxidant stress (6). The failure of the mouse models to explain non-allergic asthma phenotypes was of course not the fault of these models, but was consistent with the then unappreciated complexity and heterogeneity of asthma. Indeed, additional mouse models of asthma developed later with non-allergic triggers suggested that distinct non-allergic pathways could actually lead to asthma independent of allergic sensitization. These diverse models of asthma confirmed the idea that asthma is extremely heterogeneous, and that innate immunity could explain several asthma phenotypes (7). Such disease models identified important roles for iNKT cells as well as Type 2 and Type 3 ILCs in asthma. We describe these innate cell types and how they regulate immunity in the lungs, particularly in the development of asthma.

### Natural Killer T cells

NKT cells represent a small fraction of T-cell receptor (TCR)-expressing, thymic derived cells, and exhibit characteristics of both T cells and NK cells. NKT cells recognize glycolipid antigens presented by the non-polymorphic major histocompatibility complex class I-like molecule, called CD1d, and express the transcription factor, promyelocytic leukemia zinc finger (PLZF) (encoded by *Zbtb16*) (8). A significant fraction of NKT cells express a semi-invariant TCR, called Vα14 Jα18 in mice and Vα24 Jα18 in humans. This invariant TCR (iTCR) is highly conserved in many mammalian species, suggesting that it is a pattern recognition receptor, and that NKT cells expressing the iTCR (iNKT cells) have evolved to play an important role in the innate immune system. A role for iNKT cells in innate immunity is supported by the fact that iNKT cells rapidly produce cytokines, including interleukin-4 (IL-4), IL-5, IL-13, IL-17, and interferon-γ (IFN-γ), much more rapidly than do conventional T cells. The rapid production of cytokines by NKT cells endows this cell type with the capacity to regulate a number of different inflammatory diseases, including infectious and autoimmune diseases, inflammatory bowel disease, cancer as well as asthma.

### iNKT cells and pulmonary infectious microbes

In the lung, iNKT cells have been shown to function as adjuvants to greatly intensify immunity to several types of bacteria in mouse models of pneumonia. Mice deficient in iNKT cells have increased mortality when infected with *Streptococcus pneumoniae*, the leading cause of community-acquired pneumonia in humans, and the mortality in infected mice was associated with a reduction in neutrophils in the lungs (9). Furthermore, activation of iNKT cells in wildtype mice with iNKT cell-activating glycolipids improves bacterial clearance of *Streptococcus*, supporting the idea that iNKT cells contributed to bacterial clearance. Moreover, more recent studies demonstrated that glycolipids isolated from *Streptococcus pneumoniae* and group B *Streptococcus*, which causes neonatal sepsis and meningitis, could bind to CD1d and directly activate iNKT cells producing IFN-γ (10). iNKT cells have been shown to be activated by phosphatidylinositol mannosides from *Mycobacterium*, suggesting a role of iNKT cells in tuberculosis (11). In addition, iNKT cells producing IFN-γ accumulate in the lungs early during infection with *Chlamydophila pneumoniae* or *Pseudomonas aeruginosa*, in association with the activation of alveolar macrophages (12, 13). In these instances, chlamydial or pseudomonal antigens activate IL-12 secretion, which then activates iNKT cells in an indirect pathway (14). Finally, during influenza infection, the activation of iNKT cells enhances immunity and viral clearance (15). Mice that lack iNKT cells are more susceptible to infection, as iNKT cells block the function of inhibitory myeloid-derived suppressor cells, which normally inhibit the development of effective adaptive immunity to influenza. While adaptive immunity (including antibody production) is necessary for optimal clearance of most bacterial and viral infections in the lung, it appears that NKT cells can play a crucial role during the infection at the lung mucosal surface, as first responders, rapidly alerting and intensifying immunity to microbial pathogens.

While iNKT cells are associated with protection from infectious agents in the lung (and also outside the lung, e.g. with *Helicobacter pylori* (16), *Leishmania donovani* (17), *Bacteroides fragilis* (18), and *Borrelia burgdorferi* (19)), microbial activation of iNKT cells can also lead to lung inflammation and AHR. For example, in mice infected with Sendai virus, iNKT cells producing IL-13 mediate a chronic inflammatory process associated with AHR, by inducing IL-13 production in alveolar macrophages (20). Further, *Sphingomonas* bacteria, which frequently colonize the lungs of patients with chronic asthma (21), express glycolipids that directly activate iNKT cells (22, 23). Indeed, administration of *Sphigomonas* glycolipids directly into the lungs of mice causes AHR and inflammation (24), suggesting that the presence of *Sphigomonas* in the lungs could contribute to the development of asthma. These studies together indicate that iNKT cells sense the presence of some bacteria in the lung and play an important role in fostering inflammation and AHR.

While much of past studies examining the responses of iNKT cells to environmental agents have focused on bacteria, there is accumulating data indicating that fungi are also recognized by iNKT cells. *Aspergillus fumigatus* is a saprophytic fungus that is ubiquitous in the environment and can cause serious respiratory tract infections particularly in immunosuppressed individuals. Moreover, *Aspergillus* spores or conidia are frequently recovered from within buildings and homes, and in soil, resulting in daily respiratory exposure in most individuals and frequent allergic sensitization, particularly in patients with asthma (25, 26). In asthma, sensitization to *Aspergillus* is associated with severe disease and reduced lung function (27, 28), and with a syndrome called Severe Asthma with Fungal Sensitization (29). Furthermore, a hypersensitivity response to *Aspergillus* develops in patients with severe asthma or with cystic fibrosis, known as allergic

bronchopulmonary aspergillosis, associated with very high concentrations of serum IgE, eosinophilia, bronchiectasis, pulmonary infiltrates, and chronic severe asthma symptoms (30).

### iNKT cells regulate allergen-induced AHR

In mouse models of asthma, *Aspergillus* extracts administered into the lungs without adjuvants have been shown to very efficiently sensitize mice and cause AHR (31). *Aspergillus* rapidly induced AHR even in class $II^{-/-}$ mice that lack conventional $CD4^{+}$ helper T cells, suggesting that *Aspergillus* potently activates some innate immune compartment related to airways disease (32). Indeed, mucosal sensitization occurred through a distinct pathway, mediated by a glycosphingolipid isolated from *Aspergillus* called asperamide B. Asperamide B was found to bind to CD1d, the class I-like restriction element of iNKT cells, and directly activate pulmonary iNKT cells secreting IL-4 and IL-13. These lung iNKT cells interacted with alveolar and interstitial macrophages, which then secreted IL-33, an innate cytokine that activates mast cells, eosinophils, basophils, Th2 cells, some iNKT cells, as well as type 2 ILCs secreting IL-5 and IL-13 (discussed below). The activation of iNKT cells and the induction of IL-33 secretion by asperamide B may represent a conserved pathway at mucosal surfaces that result in Th2-biased inflammatory responses, and may help explain the high capacity of *Aspergillus* to induce AHR and cause severe hypersensitivity lung disease. In contrast, invasive tissue infection with *Aspergillus* appears to activate a different compartment of the innate immune system, mediated by dectin-1-induced IL-12 production in DCs, which then activates IFN-γ producing iNKT cells and neutrophils (33).

While pulmonary pathogens such as *Aspergillus* express glycolipids that directly activate iNKT cells, there is accumulating evidence that other environmental agents express glycolipids that directly activate mucosal iNKT cells producing IL-4, IL-5, and IL-13, and that mucosal iNKT cells play a more general role in responding to the environment. For example, in addition to *Aspergillus* as discussed above, house dust and plant pollens contain glycolipids that directly activate iNKT cells, which could intensify allergic adaptive immunity (34–36). Moreover, environmental agents such as air pollution may also activate iNKT cells, as demonstrated by exposure of mice to ozone, a major component of air pollution, which leads to the development of AHR (37). In this instance, chronic exposure to ozone activates and expands IL-17-producing iNKT cells in the lung, which can directly mediate the development of AHR. Thus, the environment may be replete with iNKT cell-activating compounds and agents that are recognized and sensed by iNKT cells, enhancing allergic sensitization, leading to the development of AHR.

As iNKT cells increase in number in the intestines and in the lungs of germ-free mice, predisposing to the development of allergic asthma (38), the reduced diversity of gut microbiota observed in asthmatic individuals (39) may result in an increase in the number of mucosal iNKT cells in infants, and increase their likelihood of developing asthma. This may explain the relationship between asthma and cesarean section delivery, in which cesarean section delivery predisposes to allergy and asthma (40), possibly by limiting or delaying the normal microbial colonization or diversity in the infant that would normally protect against asthma and allergy (41). Additional experiments in humans are needed to validate this hypothesis and to demonstrate a relationship between microbial dysbiosis, increased iNKT cell numbers, and the development of allergic asthma.

There is growing evidence, particularly in mouse models of asthma, showing that iNKT cells promote and are required for the development of AHR and airway inflammation (42–44), although their role in human asthma is less certain (45). Therefore, in addition to responding to glycolipids from fungi, pollens, house dust, and ozone, iNKT cells appear to be required to license Th2 cells to mediate AHR mice. In these studies performed in a mouse model of allergic asthma, iNKT cells producing IL-4 and IL-13 and expressing the IL-25 receptor (IL-17RB) appeared to license Th2 cells to induce AHR (43, 46). However, because functional depletion studies of iNKT cells in humans cannot be accomplished as yet, the role of iNKT cells in human asthma is controversial (42), although iNKT cells are found in increased number in the lungs of patients with asthma. In any case, the activation of iNKT cells by *Aspergillus* and other environmental allergens is very consistent with the idea that iNKT cells play an important role in regulating the development of AHR and asthma.

### iNKT cells that inhibit lung inflammation

Although most studies of iNKT cells have focused on their role in initiating and intensifying inflammation and immunity, recent studies have identified other subsets of iNKT cells that suppress immunity. Inhibitory iNKT cells were initially observed in the setting of bone marrow and allograft transplantation (47, 48) and Type 1 diabetes mellitus (49).

However, regulatory/suppressor iNKT cells found in the lung can also protect against the development of AHR and block airway inflammation (16). Lung suppressor iNKT cells have been shown to have unique characteristics, as 'double negative' (non-CD4, non-CD8), producing IFN-γ, and expanding in the lungs of very young mice when infected with influenza virus or exposed to glycolipids from *H. pylori*. These exposures in young mice then protect the mice as adults from the development of allergen-induced AHR. Suppressor iNKT cells are normally absent in the lungs of adult mice, and influenza infection in adult mice failed to expand the suppressor iNKT cells. The protective effect of early exposure to influenza or to *H. pylori* glycolipids replicates the features of the hygiene hypothesis, in which infections in young children are thought to later prevent the development of asthma and allergy. Importantly, *H. pylori* is one of the few specific infectious agents associated with protection against the development of asthma and allergy (50). The great decline in the incidence of infection with *H. pylori* over the past 3 decades secondary to improved hygiene has correlated with a great increase in the prevalence of asthma, a relationship that might be explained by activation of suppressor iNKT cells by *H. pylori* glycolipids. In any case, therapeutic methods to expand this population of suppressor iNKT cells may be useful in treating, or preventing the development of, asthma and allergy.

These studies together indicate that iNKT cells can respond rapidly to environmental microbes, allergens, and ozone, and then enhance subsequent adaptive immune responses to these agents. Although the activation of different iNKT cells subsets in the lung leads to different outcomes, these studies support the idea that iNKT cells play a critical role as innate responders and regulators of immunity in the lung.

## Innate lymphoid cell family

A growing body of evidence suggests that another related innate cell type in the lungs, ILCs, also responds rapidly to environmental signals. ILCs producing Th2 cytokines were first reported in 2001 as non-T, non-B cells that were activated and expanded *in vivo* after administration of rIL-25 (51, 52), associated with a marked increase in serum IgE, IgG1, IgA, and increased mucus production and eosinophilia in the lungs and intestinal tract. These class II$^+$ 'accessory cells', as they were initially called, were unlike any previously known cell type, and were thought to be basophil/mast cell precursors, as they expressed c-Kit but not FcεR1 (53, 54). In 2009, similar innate, non-T, non-B cells producing IL-5 and IL-13 were described in the lungs of patients with asthma (55). However, their specific functions or significance in asthma were not understood. Then in 2010, three independent groups described non-T, non-B, lymphoid cells that responded to IL-25 or IL-33 by producing large quantities of IL-5 and IL-13 (56–58). These cells, called natural helper cells, nuocytes, or type 2 innate cells, were isolated from gut-associated adipose tissue and gut-associated lymphoid tissue in mice, and were found to expand and mediate protection during helminth infection. These intestinal cells appeared to have conserved effector cell functions in innate immunity to pathogenic and non-pathogenic microorganisms, and in tissue homeostasis, repair and remodeling (56, 57, 59, 60). In addition, an immature ILC type was described that produced IL-9 before producing IL-13 and IL-5 (61). Soon after the description of these ILCs in the gut, ILCs producing IL-13 and IL-5, and others producing IL-17 were found to have important roles in the lungs, as discussed below (62–64).

As ILCs producing IL-13 and IL-5 were being defined, ILCs producing IL-17 and IL-22 were also described, called lymphoid tissue-inducer (LTi) cells and NK-22 cells (65, 66). All of these ILCs lacked antigen-specific receptors (e.g. TCRs or BCRs), but rapidly produced cytokines, at levels thought to be greater than that of T cells, in response to a wide range of innate signals, including the IL-25, IL-33, IL-12, IL-23, and IL-1β. The rapid production of cytokines by the ILCs suggested an important role of ILCs in immune regulation, though in an antigen-non-specific fashion. While the specific characteristics and features of ILCs were being characterized, a consensus nomenclature panel proposed a classification scheme based on the transcriptional regulation of ILCs, as described below (67) (*Fig. 1*).

### Type 1 innate lymphoid cells (ILC1s)

Type 1 ILCs or ILC1s are related to NK cells and require the transcription factor T-bet. However, unlike NK cells, ILC1s express little cytolytic activity, no perforin or granzyme B (68) (*Fig. 1*). ILC1s produce IFN-γ in response to IL-12 and IL-15, are present in the intestines of mice with colitis and in the intestines of patients with Crohn's disease, and amplify the pathogenic effects of CD8$^+$ T cells (68–70). In this context, ILC1s in the gut epithelium may initiate IFN-γ response against pathogens, but contribute to pathology when dysregulated (69). Whether ILC1s also function in the lungs is not yet known.

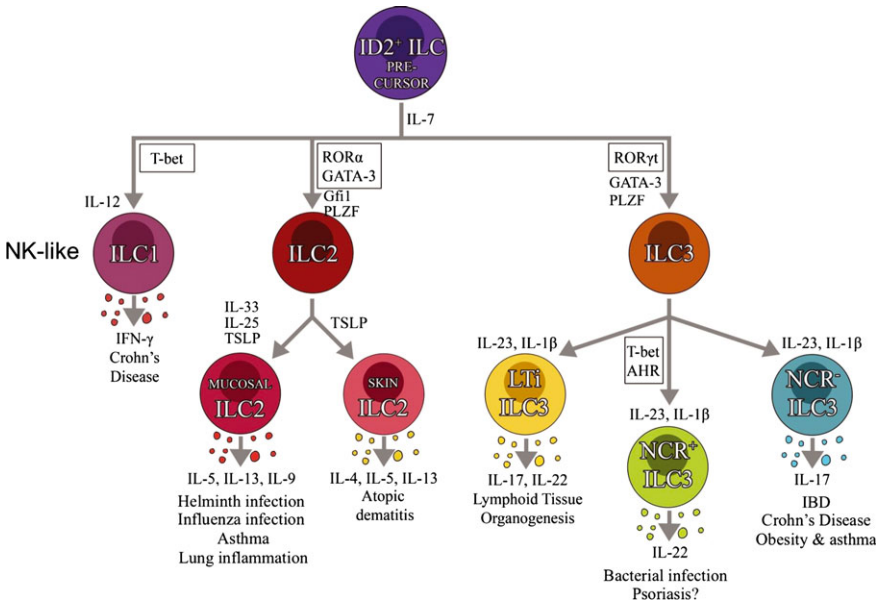


***Fig. 1.* Development and function of ILCs.** All ILCs develop from a common precursor cell characterized by expression of the transcriptional repressor inhibitor of DNA binding 2 (ID2), expression of the common cytokine receptor γ-chain (not shown), and dependence on IL-7 for development. ILC1s produce interferon-γ (IFN-γ) upon stimulation with IL-12, depend on T-bet for their development, and are involved in the pathogenesis of Crohn's disease. ILC2s depend on the transcription factors GATA3, RORα, Gfi1, and PLZF for their development. They produce IL-5, IL-13, and IL-9, although skin ILC2 cells are reported to produce IL-4, IL-5, and IL-13. ILC2s are activated by stimulation with IL-33, IL-25, and TSLP, as well as IL-2, and participate in the development of some forms of asthma, and possibly atopic dermatitis and rhinosinusitis. ILC3s require the transcription factor RORγt, GATA3, and PLZF for their development, and respond to IL-23 and IL-1β. ILC3 cells include three different subsets: LTi ILC3, $NCR^+$ ILC3, and $NCR^-$ ILC3. LTi ILC3 play important role in lymphoid tissue organogenesis and produce IL-17 and IL-22. $NCR^-$ ILC3s express CCR6, produce IL-17 and sometimes IL-22, and are critical in the development of obesity-induced asthma and inflammatory bowel disease. $NCR^+$ ILC3s produce IL-22, and also depend on the aryl hydrocarbon receptor (AHR) and T-bet for their function. Some ILC3s downregulate RORγt and transform into ILC1 cells. $NCR^+$ ILC3s may contribute to the development of psoriasis. Modified from a figure in Yu et al, J Allergy Clinc Immunol. 2014. 133:943.

### Type 2 Innate lymphoid cells (ILC2s)

The three reports that simultaneously described ILCs producing IL-5 and IL-13 described cells with slightly differing cell surface markers and function. Their similarities, including their responses to IL-25, IL-33, and TSLP and requirement for the transcription factor retinoic acid receptor-related orphan receptor α (RORα) were sufficient to name them all as type 2 ILCs or ILC2s (71). The subtle differences among the cells in the initial reports may reflect minor differences of ILC2s from different mouse strains, organs, or tissues, and with different activation states. In general however, ILC2s require the transcription factors Id2, RORα, GATA3, and Gfi1, as well as Notch signaling, and IL-7 and IL-33 for their development (72–76). In mediating anti-helminth responses, ILC2s cause eosinophilia, goblet-cell, and mast cell hyperplasia, which are also hallmarks of allergic diseases. ILC2s express CD25 (IL-2Rα), CD90 (Thy1), variable amounts of CD117 (c-Kit) and CD127 (IL-7Rα), and mediate type 2 immunological responses during helminth infection. In addition, ILC2s express variable amounts of CD278 (ICOS), ST2 (IL-33R), and IL-17RB (IL-25 receptor), and respond and expand to IL-33 IL-25 (IL-17E) and IL-2. Their role in the lung disease is currently being defined, and recent studies of lung inflammation and development of asthma are discussed below.

### Type 3 Innate lymphoid cells (ILC3s)

ILC3s require the transcription factors RORγt and GATA3 for development (77–79), and include at least three different subtypes: (i) LTi cells, required for lymphoid organogenesis and which produce IL-17 and IL-22 (80); (ii) IL-22-producing ILC3s, participating in host defense in the skin, lungs, and gut; and (iii) IL-17 producing ILC3s, which express CCR6 and are active in the gut in some forms of inflammatory bowel disease (81). IL-22-producing ILC3s depend on the aryl hydrocarbon receptor (AHR) (82) and STAT3 signaling (83), and protect against intestinal bacterial infection through several mechanisms. First, IL-22 induces anti-microbial peptide production and improves the integrity of the epithelial cell barrier. Second, IL-22-producing ILC3s enhance IgA synthesis by secreting sLTα3, which attracts helper T cells into the *lamina propria*, and by expressing

membrane-bound LTα3 (LTα1β2), which stimulates DCs to directly induce IgA synthesis (82, 84, 85). IL-17-producing ILC3s respond to IL-23 and IL-1β and may sometimes secrete IL-22 or IFN-γ. Recently, IL-17-producing ILC3s have been shown to exist in the lungs and mediate obesity-associated asthma (64), as described below.

Together, ILC1s, ILC2s, and ILC3s define a universe of cells that parallels the universe of $CD4^+$ adaptive Th cells, which includes Th1 cells (comparable to ILC1s/NK cells), Th2 cells (comparable to ILC2s) and Th17 and Th22 cells (comparable to ILC3s). Like $CD4^+$ Th cells, which arise from a common precursor cell, ILCs arise from a common precursor cell, characterized by the expression of the transcription factors Id2 (56, 86) and PLZF (87), the signature transcription factor of NKT cells. The requirement for PLZF by both NKT cells and ILCs links these two innate cell types, which rapidly produce large quantities of cytokines and which regulate mucosal immunity, particularly in the lungs. Finally, the existence of the parallel universes of adaptive and innate cells, both associated with the production of a wide variety of cytokines and regulating many forms of immunity, blurs the lines between innate and adaptive immunity.

### ILC2s in asthma

ILC2s produce the Th2 cytokines IL-5, IL-9, and IL-13, which has suggested that ILC2s might play an important role in allergic asthma (7). However, their precise role in the airways has not been as easy to define as initially thought. As ILC2s respond to IL-33, which directly causes AHR when administered into the airways (88), IL-33 receptor-deficient mice ($ST2^{-/-}$ mice) have been used to help define the role of ILC2s in the airway. While ILC2s also respond to IL-25, which has also been used to identify ILCs, IL-33 tends to provide a stronger stimulus to ILC2s than IL-25. Thus, $ST2^{-/-}$ mice fail to develop functional ILC2s (56) and also fail to develop AHR after infection with influenza, indicating an important role for ILC2s in mediating AHR associated with influenza infection (62). These studies, which were the first to demonstrate a role for ILC2s in the lungs, showed that influenza infected alveolar macrophages, which then released IL-33, that in turn activated ILC2s to expand, secrete IL-13 and IL-5, and mediate AHR. IL-33 was also released from interstitial macrophages, DCs, and possibly from iNKT cells (89). Importantly, the AHR response to influenza could occur in $Rag^{-/-}$ mice, which lack adaptive immunity but have ILCs, demonstrating that influenza-induced AHR could occur independently of adaptive immunity. Furthermore, following viral clearance, influenza-induced IL-5 production by ILC2s enhanced the accumulation of eosinophils in the airways, although early during infection, neutrophils predominated in the lungs (89).

The fact that ILC2s played an important innate role during viral respiratory infection, a trigger that causes most acute exacerbations and hospitalizations for asthma, may help to provide an immunological mechanism for viral-induced asthma. Although different respiratory viruses may activate distinct immunological pathways, previous paradigms of viral-induced asthma have focused on how viral infections intensify Th2/allergen-induced lung inflammation and AHR (90, 91). In patients with asthma however, viral infections rapidly precipitate symptoms in virtually all patients regardless of the presence of allergy, suggesting that asthma results from the rapid activation by viral infection of innate, non-Th2 mechanisms. The surprising observation that influenza could induce the development of AHR even in the absence of adaptive immunity, e.g. in $Rag2^{-/-}$ mice, is consistent with this idea. The AHR response to influenza however, depended on IL-33, its receptor, ST2 (*Il1rl1*), and ILC2s expressing c-Kit, Sca-1, Thy1.2 (CD90), and ST2 (62). Influenza-induced AHR was also dependent on IL-13, and adoptive transfer experiments demonstrated that IL-13-secreting ILC2s were capable of restoring influenza-induced AHR in $IL\text{-}13^{-/-}$ hosts. Moreover, depletion of ILC2s by treatment of $Rag2^{-/-}$ mice with anti-CD90 (Thy1) mAb to eliminate ILC2s abolished the influenza-induced AHR response. Although human rhinovirus might cause asthma by accentuating pre-existing allergic disease (92), influenza, like another virus, respiratory syncytial virus, appears to induce the development of asthma independently of allergy (93).

ILC2s may play a role in restoring lung-tissue homeostasis after influenza infection, as depletion of CD90 expressing ILC2s in $Rag1^{-/-}$ mice increased influenza-induced airway damage. ILC2s produce amphiregulin, which was required for maintaining epithelial cell integrity and improving lung function following viral infection, and may synergize with IL-22, which also enhances lung epithelial repair following influenza infection (94). Thus, ILC2s may both promote inflammatory lung disease and also restore airway epithelial cell integrity after injury. While these two functions of ILC2s may appear contradictory, the homeostatic versus the pathological role of ILC2s may be similar to the contrasting

roles of several other immune cell types. For example, Th1, Th2, and Th17 cells each have evolutionarily conserved beneficial effects (in resistance to intracellular, helminth, and fungal infections, respectively), as well as pathological effects (in causing autoimmunity or allergy). Therefore, the context in which ILC2s function may determine whether the cells are beneficial (enhancing epithelial cell integrity) or detrimental (causing airway inflammation and AHR). During helminth infection, ILC2s respond rapidly and are essential for the initial Th2-like response that enhances effective adaptive responses, associated with eosinophilia, increased mucus production, and worm expulsion. In addition, ILC2s may have evolved to respond rapidly during viral infection to repair lung injury. On the other hand, when activated in the absence of appropriate regulation, ILC2s may cause disease, such as airway inflammation and AHR. Alternatively, subsets of ILC2s may exist, with some subsets producing more IL-13 and less amphiregulin and causing airways disease, while others producing more amphiregulin and less IL-13 and primarily repairing airway injury. Further investigation into the precise characteristics of ILC2s will likely resolve this issue.

These studies demonstrate that innate IL-13 producing ILC2s play a critical role in causing AHR, in a manner independent of adaptive immunity and allergic disease. These studies help to explain how and why influenza infection rapidly causes symptoms of asthma in large numbers of patients. In addition, these studies suggest that ILC2s may be involved in asthma triggered by other respiratory viral infections. Moreover, recent studies demonstrate that ILC2s are resistant to corticosteroid treatment, particularly after exposure to TSLP (95), as discussed below. Therefore, understanding the immunobiology of ILC2s in respiratory viral infection may lead to improved therapies for virus-induced asthma, a condition that is often resistant to conventional therapies with corticosteroids.

### ILC2 in allergic airway disease

A number of studies suggest that although ILC2s may be increased in the lungs of mice with allergen-induced airways disease (96), ILC2s do not appear to be required for allergen-induced AHR. Thus, Barlow *et al.* (97), using $Il4^{+/eGFP}$ $Il13^{+/Tomato(Tom)}$ dual reporter mice, showed that after ovalbumin (OVA) sensitization and challenge, the number of ILC2s producing IL-13 greatly increased in the lung, although the majority of cells producing IL-13 were $CD4^+$ T cells. Interestingly, the majority of IL-4 producing cells in the lungs were basophils, although in the draining lymph nodes T cells produced much of the IL-4. Therefore, both T cells and innate cells (ILC2s and basophils) expand in the allergic lung and produce Th2 cytokines. However, $ST2^{-/-}$ mice had normal or near normal AHR responses after sensitization and challenge with allergen (62, 97), suggesting that ILC2 can enhance, but is not required for allergen-induced AHR.

In studies of intranasal administration of recombinant IL-25 or IL-33, IL-5- and IL-13-producing ILC2s also expanded in the lungs, bronchoalveolar lavage (BAL) fluid, and mediastinal lymph nodes (97–101). However, IL-33 affects ILC2 cells as well as Th2 cells, and treatment with blocking antibodies against ST2 reduced IL-4 and IL-13 secretion, and reduced the persistence of allergic inflammation and AHR, as measured 1 week after discontinuing allergen challenge (102). While the focus of these latter studies was on allergen-specific Th2 cells, ILC2s likely contributed to the late type 2 allergic airways disease.

#### Activation of ILC2s in the lungs

While alveolar macrophages infected with influenza produce much of the IL-33 that activates ILC2s during influenza infection, injured airway epithelial cells are thought to release the IL-33 that activate ILC2s after allergen exposure. Thus, Wilhelm *et al.* (61) found that administration into the lungs of papain, an allergen with protease activity (protease activity is often expressed by potent allergens), activated IL-9-producing ILC2s, which may represent an immature form of ILC2s. This process required IL-2 and IL-33 (but not IL-25), and blockade of IL-9 resulted in reduced expression of IL-5 and IL-13, suggesting that ILC2s may initially produce IL-9 and then mature in an autocrine fashion to produce IL-5 and IL-13. Similarly, Halim *et al.* (96) showed that protease-containing allergens damaged airway epithelial cells, which released IL-33 that in turn activated ILC2s. Thus, intranasal administration of papain to $Rag1^{-/-}$ mice, but not $Rag2^{-/-}$ $Il2rg^{-/-}$ mice (ILC2s are present in the $Rag1^{-/-}$ mice but not in the $Rag2^{-/-}$ $Il2r\gamma^{-/-}$ mice) rapidly caused lung eosinophilia, mucus hypersecretion, and elevation in BAL IL-5 and IL-13 levels (AHR was not examined). Furthermore, depletion of ILC2s in $Rag1^{-/-}$ mice by anti-CD25 mAb injection significantly reduced lung eosinophilia and mucus secretion after papain administration, and adoptive transfer of ILC2s into $Rag2^{-/-}$ $Il2r\gamma^{-/-}$ mice reconstituted these symptoms. Similar results were obtained using allergen extracts from *Alternaria alternate*, which also

have protease activity, and which induced IL-33 production and IL-25 production from airway epithelial cells driving the expansion of lung $Lin^-$ $CD25^+$ $CD44^{hi}$ ILC2s (98). These results together indicate that epithelial cells are an important early source of IL-33 and IL-25 that activate ILC2s producing IL-5, IL-9, and IL-13, and that this occurs in a T-cell-independent manner. While all of these studies suggest that ILC2s expand and are present during allergen-induced airways disease, none have shown that ILC2s are actually *required* for the development of allergen-induced AHR. Further study of this important question is necessary to more precisely understand the ILC2 requirement in allergic lung disease.

## Interaction between ILC2s and other innate and adaptive cells

As an innate cell type, ILC2s likely interact with many other cell types. First, ILC2s have been shown to synergize with Th2 cells in the gut for the expulsion of metazoan helminths (57). ILC2s respond early during helminth infection (presumably to IL-33, IL-25, and TSLP released by damaged gut epithelial cells). However, effective elimination of helminths also requires adaptive Th2 cells, which develop later during infection. Interestingly, the sustained presence of ILC2s in the gut during helminth infection required adaptive immunity (57), presumably due to the production by the adaptive cells of IL-2, which may also increase IL-9 (and IL-5 and IL-13) production by ILC2s (103). Whether this type of interaction between $CD4^+$ T cells and ILC2 cells occurs during allergen challenge is not yet clear, although it is not difficult to imagine.

### Interaction between ILC2 cells and NKT cells

As noted earlier in the section on iNKT cells, ILC2s and iNKT cells have synergistic activity in the lungs during the induction of AHR with some allergens, including *Aspergillus fumigatus* (32). The interaction between iNKT cells, airway epithelial cells, and macrophages may be mediated by OX40L and CD1d expressing antigen-presenting cells (104). In the lung, epithelial cells and macrophages may present environmental glycolipid antigens to and directly activate iNKT cells, which in turn may trigger the APC to produce IL-33. The combination of epithelial cell/macrophage and iNKT cell activation with the production of IL-33, which stimulates not only ILC2 cells, but some iNKT cells, mast cells, basophils, and eosinophils, could result in the rapid induction of AHR and inflammation (105). Therefore, this innate ILC2-iNKT cells-ST2/IL-33 pathway that is triggered by many important environmental factors/allergens [e.g. *Sphingomonas* species, *Aspergillus*, pollens, house dust, and others (19, 32, 34, 35)] appears to be clinically important not only in directly causing lung disease but also in activating the adaptive immune system (Fig. 2).

### ILC2 cells, eosinophils, and mast cells

As ILC2s produce IL-5, they also appear to control eosinophil homeostasis in the lung, small intestine, and peripheral blood, particularly in naive mice, where ILC2s represent the majority of IL-5-producing cells in the peripheral tissues. Importantly, the half-life of ILC2s is longer than that of $CD4^+$ T cells producing IL-5, and thus is more effective in maintaining serum IL-5 levels (106). Moreover, ILC2s release IL-5 in response to vasoactive intestinal peptide, which is released after feeding, which may help to explain eosinophil circadian eosinophilopoiesis, tissue accumulation, and cycling. Similar interactions between ILC2s and eosinophils occur in visceral adipose tissue, where ILC2s promote the accumulation of eosinophils and maintain alternatively activated macrophages (107).

In mice, ILC2s have been shown to be abundant in the skin and require IL-7 for survival (108). These cells constitutively produced IL-13 (and possibly IL-9, a mast cell growth factor), and interacted with cutaneous mast cells, which are known to produce IL-33 (enhancing ILC2 growth) (109). This suggests that the recognition of allergens by IgE on mast cells may lead to increased secretion of IL-33, which activates more mast cells, basophils, and eosinophils as well as ILC2s, which then secrete IL-5, further enhancing eosinophil accumulation. Thus, once allergen-specific IgE is present, in combination with allergen, this innate pathway involving mast cells/ILC2s/IL-33/IL-9 and releasing IL-13 and IL-5, could persistently drive an allergic type of inflammation, independent of conventional $CD4^+$ T cells (Fig. 2). On the other hand, this pathway might attract DCs and antigen-specific Th2 cells, and drive their expansion (Th2 cells express ST2). While the interaction between ILC2s with mast cells was noted in the skin (108), it is likely that this interaction pathway with ILC2s, mast cells, basophils, eosinophils, and Th2 cells also occurs in the lung and in the gut, in the context of asthma and food allergy.

### Corticosteroid insensitivity of ILC2s

While ILC2s appear to play a supporting role in allergic asthma (where allergen-specific Th2 cells and IgE play a

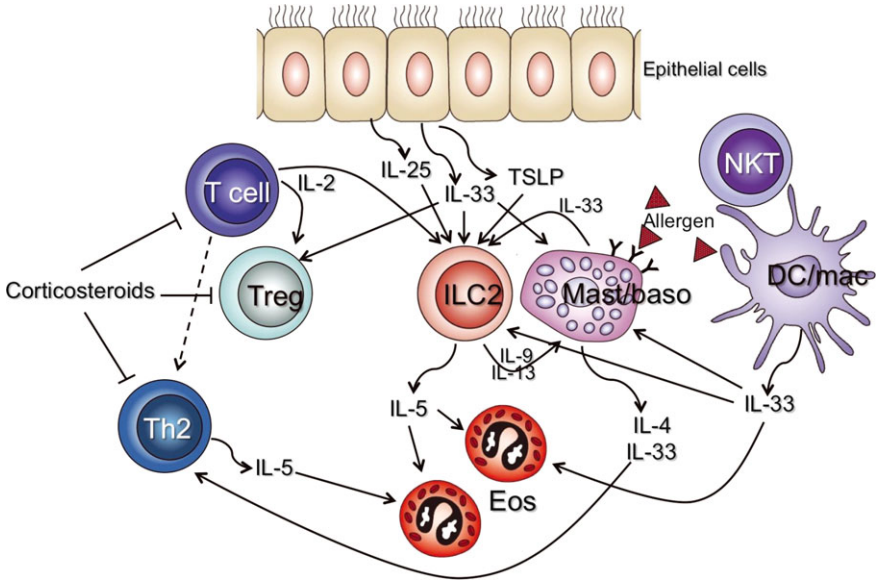


***Fig. 2. The ILC2-NKT-mast cell/basophil–eosinophil axis.*** Damaged epithelial cells can release IL25, IL-33, and TSLP, which can enhance the development and activation of ILC2s. T cells secrete IL-2, which can also enhance ILC2 development. TSLP with IL-33/IL-2 cause ILC2s to become corticosteroid resistant, in contrast to T cells, Treg cells, and Th2 cells. ILC2s secrete IL-9 and IL-13, which can activate mast cells and basophils, interact with mast cells. ILC2 also secrete IL-5, which can activate eosinophils. Mast cell activation from allergen exposure results in increased secretion of cytokines from mast cells and basophils (IL-4, IL-13, IL-33). NKT cells respond to glycolipids from some allergens, and can then activate DCs and macrophages to produce IL-33, which activates multiple cell types in the network. NKT cells can also secrete IL-4, IL-5, IL-13, and IL-33. IL-33 from all sources can also activate Th2 cells. Thus, allergen exposure/epithelial cell injury results in the activation of a tight network that self amplifies and sustains an allergic Type 2 inflammatory response.

dominant role), there may be situations during allergic diseases when ILC2s might play a more prominent role. A recent study showed that ILC2s become corticosteroid resistant after exposure to IL-33 plus TSLP (or plus IL-7 or IL-2) (95). This situation may arise in allergic asthma or atopic dermatitis during treatment with corticosteroids, which may eliminate Th2 cells, as they are particularly sensitive to corticosteroids. In the lungs and skin, in asthma and in atopic dermatitis, respectively, TSLP is highly expressed, allowing ILC2s, but not Th2 cells to become resistant to corticosteroid treatment. The insensitivity of TSLP-exposed ILC2s producing IL-13 and IL-5 may thus explain the frequent clinical scenario of corticosteroid-resistant allergic asthma and atopic dermatitis. In such situations, therapies that target ILC2s might produce improved clinical outcomes.

### ILC2 cells in human disease

The initial studies of ILC2s were all performed in mice, as has been the case with many other immune cell types (e.g. Th1, Th2, Th17 cells, DCs, etc.). As with many of the observations of other cell types in mice, the murine studies are being replicated in humans, suggesting an important role for ILC2s in humans, particularly in the gut, respiratory tract, and skin. As mentioned earlier, IL-13 and IL-5 producing non-T non-B cells with characteristics of ILC2s were found in the sputum of asthmatic but not normal subjects (55). These non-T non-B cells expressed CD34, responded to TSLP and IL-33, and increased in number after specific allergen inhalation challenge, as predicted by murine studies of ILC2s, although these cells were not further characterized. In addition, Barnig (110) confirmed the presence of ILC2-like cells in human lungs expressing c-Kit and CD161 but not FcεR1, and responding to IL-25, IL-33, and prostaglandin D2. Airway epithelial cells are a likely source of the IL-33, IL-25, and TSLP in humans, although airway smooth muscle cells have also been shown to produce IL-33 in severe asthma (111).

Other recent studies have identified human ILC2s in several tissues: fetal gut identified as $Lin^-$, IL-7Rα $(CD127)^+$ cells expressing CRTH2 and CD161 (112); in the pleural effusion of patients with primary spontaneous pneumothorax, associated with the presence of IL-5, IL-33, and TSLP (113); and in the skin of patients with atopic dermatitis, identified as $Lin^-$ $CD127^+$ cells responsive to TSLP and not IL-33 or IL-25 (114) or as responsive to all three cytokines (115). These reports suggest that the specific cell surface markers and function of ILC2s may vary depending on the activation state and tissue site (e.g. lung, gut, or skin), similar

to studies of murine ILC2s. Finally, ILC2s have been found in nasal polyp tissue from patients with chronic rhinosinusitis (112). As TSLP is increased in nasal polyps of patients with chronic rhinosinusitis (116) and as IL-33 and ST2 are increased in the serum and tissue of patients with allergic rhinitis (117), ILC2s may play an important role in humans with allergic rhinitis. Finally, genome-wide association studies have identified the genes for IL-33, ST2, RORα, and TSLP as important susceptibility genes for human asthma (118, 119). Because the products of these genes are all directly involved in the function of ILC2 cells, these genetic studies strongly suggest that ILC2s play an important role in humans, not only in the respiratory tract, but also in the skin and gut.

## ILC1s and ILC3s and asthma

### ILC1s and asthma

ILC1s or NK cells have not been studied extensively in the context of asthma, in part because the tools for specifically ablating ILC1s or NK cells are limited. However, more than a decade ago, Korsgren *et al.* (120) showed that by depleting both NK and NKT cells in mice with an anti-NK1.1 antibody, eosinophilic lung inflammation was greatly reduced, as was allergen-specific IgE and IgG, IL-5, and IL-12 production in the lung. Because NKT cell-deficient mice had near normal allergen-induced airway eosinophilia, these investigators concluded that NK cells must be the critical component that enhanced allergen-induced airway inflammation. On the other hand, others have since demonstrated that NKT cells actually enhance airway eosinophilia and are required for allergen-induced AHR. These results suggest that Korsgren's conclusions may need reconsideration, and that NK cells may not augment allergic airways disease, as originally thought. In fact, more recent examination of NK cells taken from the lungs of patients with asthma suggested that NK cells promoted eosinophil apoptosis and functioned to inhibit eosinophilic airway inflammation (110). Therefore, the specific role of NK/ILC1s in asthma needs further study.

### ILC3 cells and asthma

The role of IL-17A producing ILC3s in asthma must of necessity relate to a role for IL-17A in asthma, which has been controversial, as IL-17A can either inhibit or worsen allergic asthma (37, 121). Recent studies however, indicate that IL-17 can directly cause AHR (122, 123), though the source of IL-17 in the lungs has been assumed to be Th17 cells or γδ T cells.

Given that IL-17 might be pathogenic in airways disease and that IL-17A production is increased in obesity, a role for ILC3s producing IL-17A in AHR associated with obesity has been recently proposed (64). Obesity, which is associated with type 2 diabetes mellitus, cardiovascular disease, liver disease, and some forms of cancer (124), is also a major risk factor for the development of asthma (125–127), particularly with a severe, therapy-resistant form distinct from allergic asthma (128). Thus, in a mouse model, Kim *et al.* (64) demonstrated that mice made obese by treatment with a high fat diet for 12 weeks spontaneously developed AHR, associated with a significant increase in IL-17 producing cells in their lungs, the majority of which were $Lin^-$ $Thy1.2^+$ $Scal\text{-}1^+$ ILC3s. Unlike ILC2s, the ILC3s were $ROR\gamma t^+$ $CD44^+$ $CCR6^+$ and did not make IL-13 (or IL-17F or IL-22). Moreover, $Rag^{-/-}$ mice treated with a high fat diet also became obese and developed AHR, associated with an increase in IL-17-producing ILC3s in their lungs, indicating that this pathway for AHR could occur in the absence of adaptive immunity. IL-17 was required for the obesity-induced AHR, as $Il17^{-/-}$ mice fed the high fat diet failed to develop AHR, even though these mice became obese. The development of IL-17 producing ILC3s in the obese mice required the NLRP3 inflammasome, as $Nlrp3^{-/-}$ mice on the high fat diet became obese, but did not develop AHR (64).

The NLRP3 infammasome is known to be activated in obesity by high glucose and the fatty acid palmitate, resulting in NLRP3-dependent IL-1β production, particularly in adipose tissue macrophages, which convert from M2 to M1 macrophages (129). Similar M2 to M1 conversion of lung macrophages occurs in obese mice, resulting in elevated lung production of IL-1β. Furthermore, blockade of IL-1β signaling by treatment of the obese mice with an IL-1R antagonist (anakinra) for 7 days abrogated development of AHR and greatly decreased the number of IL-17 producing lung ILC3s. IL-1β was responsible for the development of IL-17 producing ILC3s, because administration of IL-1β to $Rag^{-/-}$ mice or to $Nlrp3^{-/-}$ mice directly induced AHR (64), associated with a great increase in IL-17-producing ILC3s in the lungs. Further, adoptive transfer of IL-17 producing ILC3s to $Rag2^{-/-}$ $Il2r\gamma^{-/-}$ mice, restored IL-1β-induced AHR, indicating that IL-17 producing ILC3s by themselves could induce AHR.

These studies together showed that ILC3s could play a critical role in obesity-associated asthma. A role for ILC3s in humans was suggested by examination of the BAL fluid

from a small group of patients with asthma, which demonstrated the presence of IL-17-producing ILC3s, particularly in patients with more severe asthma (64). These studies were the first to demonstrate that ILC3s are present in the lungs of humans and suggest that ILC3s might indeed play an important role in at least some forms of human asthma.

Finally, these studies suggest that metabolic and nutritional factors greatly affect the development of innate immunity. Indeed, ILC3s expand in response to other nutritional factors, such as vitamin A. Thus, vitamin A deficiency resulted in a significant reduction in IL-22- and IL-17-producing intestinal ILC3s (130). Interestingly, vitamin A deficiency also resulted in a great increase in ILC2s. As a result, vitamin A deficiency caused susceptibility to intestinal bacterial infection, but resistance to intestinal helminth infection, extending the idea that nutrition greatly affects ILC homeostasis.

## Concluding remarks

Studies of iNKT cells and ILCs in asthma over the past several years have changed our understanding of the principles of immune regulation and provided specific mechanisms for the development of several forms of non-allergic asthma. iNKT cells and ILCs are related in that they both rapidly produce a broad array of cytokines, rivaling those of adaptive T cells, and both utilize the transcription factor PLZF. Moreover, iNKT cells, ILC2s, and ILC3s all respond to environmental triggers important in clinical asthma. While iNKT cells and ILCs can function independently of adaptive immunity, it is likely that these innate cells also function in conjunction with adaptive immunity to shape immunity to environmental triggers, including specific allergens, infectious and commensal microbes, and dietary/nutritional effects. Future studies of iNKT cells and ILCs are also likely to lead to improved therapies for allergic diseases and asthma.

## References


1. Hilty M, et al. Disordered microbial communities in asthmatic airways. PLoS One 2010;**5**:e8578.
2. Wenzel S, et al. Dupilumab in persistent asthma with elevated eosinophil levels. N Engl J Med 2013;**368**:2455–2466.
3. Corren J, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med 2011;**365**:1088–1098.
4. Nair P, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med 2009;**360**:985–993.
5. Robinson DS, et al. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 1992;**326**:298–304.
6. Wenzel S, Holgate ST. The mouse trap: it still yields few answers in asthma. Am J Respir Crit Care Med 2006;**174**:1173–1176; discussion 1176–1178.
7. Kim HY, DeKruyff RH, Umetsu DT. The many paths to asthma: phenotype shaped by innate and adaptive immunity. Nat Immunol 2010;**11**:577–584.
8. Savage AK, et al. The transcription factor PLZF directs the effector program of the NKT cell lineage. Immunity 2008;**29**:391–403.
9. Kawakami K, et al. Critical role of Valpha14+ natural killer T cells in the innate phase of host protection against Streptococcus pneumoniae infection. Eur J Immunol 2003;**33**:3322–3330.
10. Kinjo Y, et al. Invariant natural killer T cells recognize glycolipids from pathogenic Gram-positive bacteria. Nat Immunol 2011;**12**:966–974.
11. Fischer K, et al. Mycobacterial phosphatidylinositol mannoside is a natural antigen for CD1d-restricted T cells. Proc Natl Acad Sci USA 2004;**101**:10685–10690.
12. Nieuwenhuis EE, et al. CD1d-dependent macrophage-mediated clearance of Pseudomonas aeruginosa from lung. Nat Med 2002;**8**:588–593.
13. Joyee AG, Qiu H, Wang S, Fan Y, Bilenki L, Yang X. Distinct NKT cell subsets are induced by different Chlamydia species leading to differential adaptive immunity and host resistance to the infections. J Immunol 2007;**178**:1048–1058.
14. Brigl M, Bry L, Kent SC, Gumperz JE, Brenner MB. Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. Nat Immunol 2003;**4**:1230–1237.
15. De Santo C, et al. Invariant NKT cells reduce the immunosuppressive activity of influenza A virus-induced myeloid-derived suppressor cells in mice and humans. J Clin Invest 2008;**118**:4036–4048.
16. Chang YJ, et al. Influenza infection in suckling mice expands an NKT cell subset that protects against airway hyperreactivity. J Clin Invest 2011;**121**:57–69.
17. Ishikawa H, et al. CD4(+) v(alpha)14 NKT cells play a crucial role in an early stage of protective immunity against infection with Leishmania major. Int Immunol 2000;**12**:1267–1274.
18. Wieland Brown LC, et al. Production of alpha-galactosylceramide by a prominent member of the human gut microbiota. PLoS Biol 2013;**11**:e1001610.
19. Kinjo Y, et al. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. Nat Immunol 2006;**7**:978–986.
20. Kim EY, et al. Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease. Nat Med 2008;**14**:633–640.
21. Huang YJ, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. J Allergy Clin Immunol 2011;**127**:372–381 e371–373.
22. Kinjo Y, et al. Recognition of bacterial glycosphingolipids by natural killer T cells. Nature 2005;**434**:520–525.
23. Mattner J, et al. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature 2005;**434**:525–529.
24. Meyer EH, et al. Glycolipid activation of invariant T cell receptor+ NK T cells is sufficient to induce airway hyperreactivity independent of conventional CD4+ T cells. Proc Natl Acad Sci USA 2006;**103**:2782–2787.
25. O'Connor GT, et al. Airborne fungi in the homes of children with asthma in low-income urban communities: the Inner-City asthma study. J Allergy Clin Immunol 2004;**114**:599–606.
26. Shelton BG, Kirkland KH, Flanders WD, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl Environ Microbiol 2002;**68**:1743–1753.
27. Fairs A, et al. IgE sensitization to Aspergillus fumigatus is associated with reduced lung function in asthma. Am J Respir Crit Care Med 2010;**182**:1362–1368.
28. Menzies D, Holmes L, McCumesky G, Prys-Picard C, Niven R. Aspergillus sensitization is associated with airflow limitation and bronchiectasis in severe asthma. Allergy 2011;**66**:679–685.
29. Agarwal R. Severe asthma with fungal sensitization. Curr Allergy Asthma Rep 2011;**11**:403–413.
30. Greenberger PA. Allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 2002;**110**:685–692.
31. Grunig G, Corry DB, Leach MW, Seymour BW, Kurup VP, Rennick DM. Interleukin-10 is a

natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. J Exp Med 1997;**185**:1089–1099.
32. Albacker LA, et al. Invariant natural killer T cells recognize a fungal glycosphingolipid that can induce airway hyperreactivity. Nat Med 2013;**19**:1297–1304.
33. Cohen NR, et al. Innate recognition of cell wall beta-glucans drives invariant natural killer T cell responses against fungi. Cell Host Microbe 2011;**10**:437–450.
34. Wingender G, et al. Invariant NKT cells are required for airway inflammation induced by environmental antigens. J Exp Med 2011;**208**:1151–1162.
35. Agea E, et al. Human CD1-restricted T cell recognition of lipids from pollens. J Exp Med 2005;**202**:295–308.
36. Scanlon ST, et al. Airborne lipid antigens mobilize resident intravascular NKT cells to induce allergic airway inflammation. J Exp Med 2011;**208**:2113–2124.
37. Pichavant M, et al. Ozone exposure in a mouse model induces airway hyperreactivity that requires the presence of natural killer T cells and IL-17. J Exp Med 2008;**205**:385–393.
38. Olszak T, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science 2012;**336**:489–493.
39. Ege MJ, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med 2011;**364**:701–709.
40. Thavagnanam S, Fleming J, Bromley A, Shields MD, Cardwell CR. A meta-analysis of the association between Caesarean section and childhood asthma. Clin Exp Allergy 2008;**38**:629–633.
41. Fujimura KE, et al. House dust exposure mediates gut microbiome Lactobacillus enrichment and airway immune defense against allergens and virus infection. Proc Natl Acad Sci USA 2014;**111**:805–810.
42. Matangkasombut P, Pichavant M, Dekruyff RH, Umetsu DT. Natural killer T cells and the regulation of asthma. Mucosal Immunol 2009;**2**:383–392.
43. Akbari O, et al. Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. Nat Med 2003;**9**:582–588.
44. Akbari O, et al. CD4+ invariant T-cell-receptor+ natural killer T cells in bronchial asthma. N Engl J Med 2006;**354**:1117–1129.
45. Thomas SY, Chyung YH, Luster AD. Natural killer T cells are not the predominant T cell in asthma and likely modulate, not cause, asthma. J Allergy Clin Immunol 2010;**125**:980–984.
46. Stock P, Lombardi V, Kohlrautz V, Akbari O. Induction of airway hyperreactivity by IL-25 is dependent on a subset of invariant NKT cells expressing IL-17RB. J Immunol 2009;**182**:5116–5122.
47. Higuchi M, et al. Immune tolerance to combined organ and bone marrow transplants after fractionated lymphoid irradiation involves regulatory NK T cells and clonal deletion. J Immunol 2002;**169**:5564–5570.
48. Seino KI, et al. Requirement for natural killer T (NKT) cells in the induction of allograft tolerance. Proc Natl Acad Sci USA 2001;**98**:2577–2581.
49. Lehuen A, et al. Overexpression of natural killer T cells protects Valpha14- Jalpha281 transgenic nonobese diabetic mice against diabetes. J Exp Med 1998;**188**:1831–1839.
50. Matricardi PM, et al. Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. BMJ 1997;**314**:999–1003.
51. Fort MM, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity 2001;**15**:985–995.
52. Fallon PG, et al. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. J Exp Med 2006;**203**:1105–1116.
53. Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. Immunity 2004;**20**:267–277.
54. Saenz SA, et al. IL25 elicits a multipotent progenitor cell population that promotes T(H)2 cytokine responses. Nature 2010;**464**:1371–1376.
55. Allakhverdi Z, et al. CD34+ hemopoietic progenitor cells are potent effectors of allergic inflammation. J Allergy Clin Immunol 2009;**123**:472–478.
56. Moro K, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+) Sca-1(+) lymphoid cells. Nature 2010;**463**:540–544.
57. Neill DR, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature 2010;**464**:1367–1370.
58. Price AE, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. Proc Natl Acad Sci USA 2010;**107**:11489–11494.
59. Buonocore S, et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. Nature 2010;**464**:1371–1375.
60. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. Nat Immunol 2011;**12**:21–27.
61. Wilhelm C, et al. An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. Nat Immunol 2011;**12**:1071–1077.
62. Chang Y-J, et al. Innate lymphoid cells mediate influenza-induced airway hyperreactivity independent of adaptive immunity. Nat Immunol 2011;**12**:631–638.
63. Monticelli L, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. Nat Immunol 2011;**12**:1045–1054.
64. Kim H, et al. IL-17 producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity. Nat Med 2014;**20**:54–61.
65. Eberl G, Marmon S, Sunshine MJ, Rennert PD, Choi Y, Littman DR. An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. Nat Immunol 2004;**5**:64–73.
66. Cella M, et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. Nature 2009;**457**:722–725.
67. Bernink J, Mjosberg J, Spits H. Th1- and Th2-like subsets of innate lymphoid cells. Immunol Rev 2013;**252**:133–138.
68. Bernink JH, et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nat Immunol 2013;**14**:221–229.
69. Fuchs A, et al. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN-gamma-producing cells. Immunity 2013;**38**:769–781.
70. Geremia A, et al. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. J Exp Med 2011;**208**:1127–1133.
71. Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. Annu Rev Immunol 2012;**30**:647–675.
72. Zhou L. Striking similarity: GATA-3 regulates ILC2 and Th2 cells. Immunity 2012;**37**:589–591.
73. 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**:463–474.
74. Wong SH, et al. Transcription factor RORalpha is critical for nuocyte development. Nat Immunol 2012;**13**:229–236.
75. Spooner CJ, et al. Specification of type 2 innate lymphocytes by the transcriptional determinant Gfi1. Nat Immunol 2013;**14**:1229–1236.
76. Hoyler T, et al. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. Immunity 2012;**37**:634–648.
77. Sanos SL, Vonarbourg C, Mortha A, Diefenbach A. Control of epithelial cell function by interleukin-22-producing RORgammat+ innate lymphoid cells. Immunology 2011;**132**:453–465.
78. Possot C, et al. Notch signaling is necessary for adult, but not fetal, development of RORgammat (+) innate lymphoid cells. Nat Immunol 2011;**12**:949–958.
79. Serafini N, et al. Gata3 drives development of RORgammat+ group 3 innate lymphoid cells. J Exp Med 2014;**211**:199–208.
80. Sonnenberg GF, Monticelli LA, Elloso MM, Fouser LA, Artis D. CD4(+) lymphoid tissue-inducer cells promote innate immunity in the gut. Immunity 2011;**34**:122–134.
81. Coccia M, et al. IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4(+) Th17 cells. J Exp Med 2012;**209**:1595–1609.
82. Qiu J, et al. Group 3 innate lymphoid cells inhibit T-cell-mediated intestinal inflammation through aryl hydrocarbon receptor signaling and

regulation of microflora. Immunity 2013;**39**:386–399.
83. Guo X, et al. Induction of innate lymphoid cell-derived interleukin-22 by the transcription factor STAT3 mediates protection against intestinal infection. Immunity 2014;**40**:25–39.
84. Sonnenberg GF, et al. Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. Science 2012;**336**:1321–1325.
85. Kruglov AA, et al. Nonredundant function of soluble LTalpha3 produced by innate lymphoid cells in intestinal homeostasis. Science 2013;**342**:1243–1246.
86. Satoh-Takayama N, et al. IL-7 and IL-15 independently program the differentiation of intestinal CD3-NKp46+ cell subsets from Id2-dependent precursors. J Exp Med 2010;**207**:273–280.
87. Constantinides MG, McDonald BD, Verhoef PA, Bendelac A. A committed precursor to innate lymphoid cells. Nature 2014. doi: 10.1038/nature13047. (in press)
88. Kondo Y, et al. Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. Int Immunol 2008;**20**:791–800.
89. Gorski SA, Hahn YS, Braciale TJ. Group 2 innate lymphoid cell production of IL-5 is regulated by NKT cells during influenza virus infection. PLoS Pathog 2013;**9**:e1003615.
90. Bartlett NW, et al. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. Nat Med 2008;**14**:199–204.
91. Holt PG, Strickland DH, Sly PD. Virus infection and allergy in the development of asthma: what is the connection? Curr Opin Allergy Clin Immunol 2012;**12**:151–157.
92. Jackson DJ, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. Am J Respir Crit Care Med 2012;**185**:281–285.
93. Blanken MO, et al. Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. N Engl J Med 2013;**368**:1791–1799.
94. Pociask DA, et al. IL-22 is essential for lung epithelial repair following influenza infection. Am J Pathol 2013;**182**:1286–1296.
95. Kabata H, et al. Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation. Nat Commun 2013;**4**:2675.
96. 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**:451–463.
97. Barlow JL, et al. Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. J Allergy Clin Immunol 2012;**129**:191–198 e191–194.
98. Bartemes KR, Iijima K, Kobayashi T, Kephart GM, McKenzie AN, Kita H. IL-33-responsive lineage-CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. J Immunol 2012;**188**:1503–1513.
99. Schmitz J, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005;**23**:479–490.
100. Hurst SD, et al. New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. J Immunol 2002;**169**:443–453.
101. Klein Wolterink RG, et al. Pulmonary innate lymphoid cells are major producers of IL-5 and IL-13 in murine models of allergic asthma. Eur J Immunol 2012;**42**:1106–1116.
102. Kearley J, Buckland KF, Mathie SA, Lloyd CM. Resolution of allergic inflammation and airway hyperreactivity is dependent upon disruption of the T1/ST2-IL-33 pathway. Am J Respir Crit Care Med 2009;**179**:772–781.
103. Veldhoen M, et al. Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol 2008;**9**:1341–1346.
104. Damayanti T, et al. Serial OX40 engagement on CD4+ T cells and natural killer T cells causes allergic airway inflammation. Am J Respir Crit Care Med 2010;**181**:688–698.
105. Kim H, et al. Innate lymphoid cells responding to IL-33 mediate airway-hyperreactivity independent of adaptive immunity. J Allergy Clin Immunol 2012;**129**:216–227.
106. Nussbaum JC, et al. Type 2 innate lymphoid cells control eosinophil homeostasis. Nature 2013;**502**:245–248.
107. Molofsky AB, et al. Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. J Exp Med 2013;**210**:535–549.
108. Roediger B, et al. Cutaneous immunosurveillance and regulation of inflammation by group 2 innate lymphoid cells. Nat Immunol 2013;**14**:564–573.
109. Hsu CL, Neilsen CV, Bryce PJ. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. PLoS One 2010;**5**:e11944.
110. Barnig C, et al. Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. Sci Transl Med 2013;**5**:174ra126.
111. Prefontaine D, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. J Immunol 2009;**183**:5094–5103.
112. Mjosberg JM, 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**:1055–1062.
113. Kwon BI, Hong S, Shin K, Choi EH, Hwang JJ, Lee SH. Innate type 2 immunity is associated with eosinophilic pleural effusion in primary spontaneous pneumothorax. Am J Respir Crit Care Med 2013;**188**:577–585.
114. Kim BS, et al. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. Sci Transl Med 2013;**5**:170ra116.
115. Salimi M, et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. J Exp Med 2013;**210**:2939–2950.
116. Nagarkar DR, et al. Thymic stromal lymphopoietin activity is increased in nasal polyps of patients with chronic rhinosinusitis. J Allergy Clin Immunol 2013;**132**:593–600 e512.
117. 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**:218–228.
118. Moffatt MF, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010;**363**:1211–1221.
119. Hirota T, et al. Genome-wide association study identifies three new susceptibility loci for adult asthma in the Japanese population. Nat Genet 2011;**43**:893–896.
120. Korsgren M, et al. Natural killer cells determine development of allergen-induced eosinophilic airway inflammation in mice. J Exp Med 1999;**189**:553–562.
121. Schnyder-Candrian S, et al. Interleukin-17 is a negative regulator of established allergic asthma. J Exp Med 2006;**203**:2715–2725.
122. Kudo M, et al. IL-17A produced by alphabeta T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction. Nat Med 2012;**18**:547–554.
123. McKinley L, et al. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. J Immunol 2008;**181**:4089–4097.
124. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med 2012;**18**:363–374.
125. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012;**18**:716–725.
126. Holguin F, et al. Obesity and asthma: an association modified by age of asthma onset. J Allergy Clin Immunol 2011;**127**:1486–1493, e1482.
127. Camargo CA, Jr., Weiss ST, Zhang S, Willett WC, Speizer FE. Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. Arch Intern Med 1999;**159**:2582–2588.
128. Moore WC, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010;**181**:315–323.
129. Wen H, et al. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat Immunol 2011;**12**:408–415.
130. Spencer SP, et al. Adaptation of innate lymphoid cells to a micronutrient deficiency promotes type 2 barrier immunity. Science 2014;**343**:432–437.