

Innate lymphoid cells in the airways

Jennifer A. Walker and Andrew McKenzie

MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

The airways, similar to other mucosal surfaces, are continuously exposed to the outside environment and a barrage of antigens, allergens, and microorganisms. Of critical importance therefore is the ability to mount rapid and effective immune responses to control commensal and pathogenic microbes, while simultaneously limiting the extent of these responses to prevent immune pathology and chronic inflammation. The function of the adaptive immune response in controlling these processes at mucosal surfaces has been well documented but the important role of the innate immune system, particularly the recently identified family of innate lymphoid cells, has only lately become apparent. In this review, we give an overview of the innate lymphoid cells that exist in the airways and examine the evidence pertaining to their emerging roles in airways immunity, inflammation, and homeostasis.

Keywords: Airways • Asthma • Cytokines • Innate immunity

Introduction

Innate lymphoid cells (ILCs) represent a recently identified family of lymphoid cells that play diverse roles in lymphoid tissue formation, immunity, inflammation, and tissue remodeling [1]. These cells lack specific, rearranged antigen receptors, and secrete cytokines to influence immune responses and remodeling of surrounding tissue. ILCs display heterogeneity with respect to their location, cytokine production, and effector functions but are unified by their lymphoid origin and requirement for the transcription factor Id2 during development. To a large extent, the repertoire of ILCs parallels that of the known subsets of T-helper (Th) cells, namely Th1, Th2, and Th17 and, while lineage relationships are not well understood, transcription factors required for the development of each specific ILC subtype have recently been identified (Fig. 1).

Natural killer (NK) cells represent the prototypic ILCs and, through their secretion of IFN- γ and their cytolytic capacity, serve as effector lymphocytes in antiviral and antitumor immunity [2]. NK cells are dependent on the cytokine IL-15 for their development and also require the transcription factor E4BP4/NF-IL3 [3, 4]. More recently, a second family of ILCs has been described

that depends upon the expression of *Rorc*, which encodes the transcription factor ROR γ t [5]. Lymphoid tissue-inducer (LTi) cells were the first of this family to be identified and are required for lymphoid organogenesis during development, and repair and remodeling of lymphoid tissues in the adult [6]. At least two other ROR γ t-dependent ILC subsets have since been identified, namely ILC17 and ILC22, which secrete the cytokines IL-17A and IL-22, respectively. ILC17 cells have been reported mainly in the intestinal tract where they become activated by IL-23 to drive inflammatory conditions of the intestine [7]. ILC22 cells are also present in the intestinal tract where they mediate early protective immune responses to enteric pathogens [8]. A third subset of LTi-like ILCs has also been identified, which simultaneously produces both IL-17 and IL-22 [9]. However, it remains to be determined whether the dual-producing ILC17/22 population represents a distinct ILC subset or merely reflects plasticity of the LTi-like lineage.

A further branch of the ILC family, identified recently by several groups, is characterized by the secretion of type-2 cytokines, including IL-13, IL-5, and IL-6. These cells were initially described as an IL-25 regulated, non-B, non-T cell population that provides an early source of type-2 cytokines during helminthic infection [10, 11]. Now variously termed nuocytes [12], natural helper cells (NHCs) [13], and innate helper (Ih2) cells [14], these cells have been defined further by their lack of all conventional lineage markers and expression of the molecules ICOS, CD90, ST2, CD25, and CD127, the latter three being subunits of the cytokine receptors

Correspondence: Dr. Andrew McKenzie
e-mail: anm@mrc-lmb.cam.ac.uk

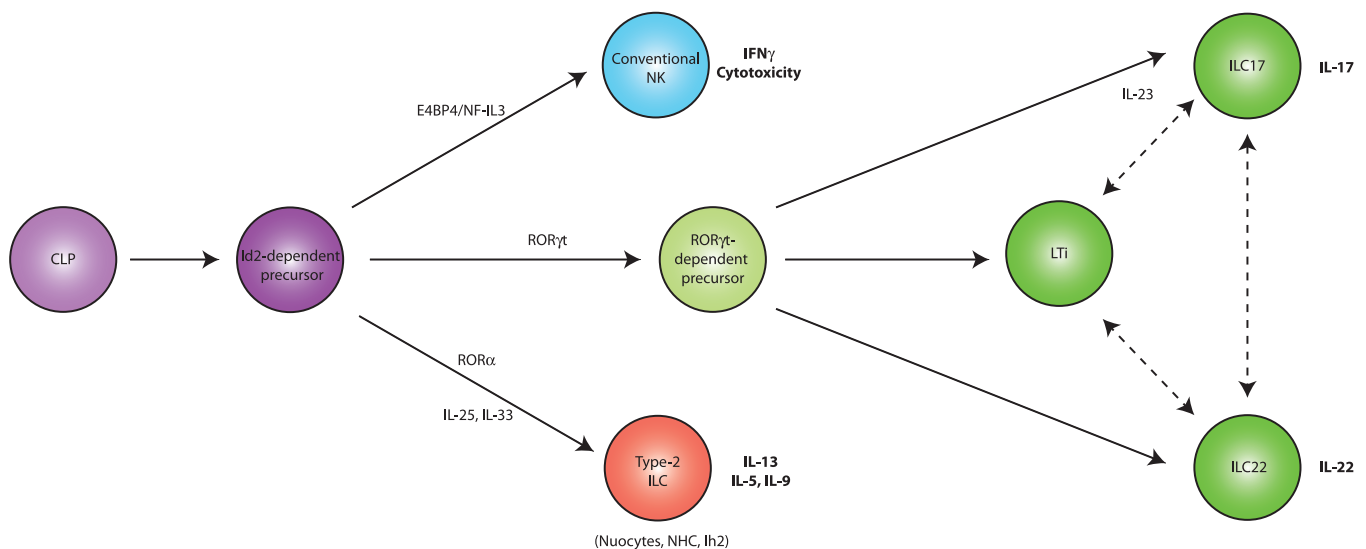


Figure 1. The innate lymphoid cell family. Distinct members of the innate lymphoid cell (ILC) family develop from common lymphoid progenitors (CLPs) and require the transcription factor, Id2. Upon expression of lineage-specific transcription factors, cells are induced to differentiate into the conventional NK, RORγt-dependent and type-2 branches of the ILC family. These cells secrete a characteristic set of cytokines that are appropriate to their immunological function.

for IL-33, IL-2, and IL-7, respectively. In response to IL-25 and IL-33, these cells are induced to secrete type-2 cytokines, notably IL-13 and IL-5, and are essential for the prompt induction of type-2 immune responses and efficient expulsion of helminthic parasites in vivo [10, 12–15]. Unlike LT_i-like cells, these ILCs do not require RORγt for their development but instead require the transcription factor RORα ([16] and our unpublished observations). Only minor phenotypic differences have been identified between nuocytes, NHCs and Ih2 cells, (e.g. variable c-Kit expression) and these can most probably be ascribed to variation in their location and maturational state. We therefore propose that while distinct subsets of type-2 cytokine secreting ILCs may well exist, for practical purposes they can be grouped collectively as “type-2 ILCs”.

The ILC subsets described above have predominantly been studied in the context of the gut mucosa and their critical and diverse roles in controlling intestinal immunity and inflammation are becoming increasingly apparent. At present, however, little is known regarding the existence of ILCs at other mucosal surfaces, such as the airways, although hints as to the critical roles ILCs might play in mediating immunity and tissue homeostasis at these sites are beginning to emerge.

Asthma, lung inflammation, and airways hyperreactivity

Allergic asthma is a chronic inflammatory disease of the airways, which occurs as a consequence of inappropriate immunological responses to environmental antigens. It is characterized by chronic airway inflammation and airway hyperreactivity (AHR), resulting in bronchoconstriction, mucus secretion, and significant morbidity. Allergic asthma is typified by a type-2 immune response

involving eosinophilia, elevated concentrations of IgE, mucus hypersecretion, and goblet cell hyperplasia. Animal models of allergic asthma have highlighted the importance of type-2 cytokines in driving these responses and a role for Th2 cells has been well established [17–20]. Evidence suggests that the immune response is initiated at the mucosal surface, where epithelial cell cytokines such as IL-25, IL-33, and TSLP (thymic stromal lymphopoietin) have been implicated in the recruitment and activation of type-2 immune cells [21]. Some of the features of allergic asthma, such as AHR and the inflammatory cell infiltrate, are mimicked in models of nonallergic asthma, which can be induced by viruses or other environmental factors, such as air pollution.

Type-2 ILCs in allergic asthma

While the function of the adaptive immune system in allergic asthma has been well characterized, the role of the innate immune system has only recently advanced to the forefront of asthma research. Mounting evidence suggests that the initial phase of a type-2 immune response occurs independently of adaptive immunity, since IL-33 or IL-25 can initiate type-2 cytokine production in *Rag2*^{−/−} mice, accompanied by eosinophilic lung inflammation and AHR ([22, 23] and our unpublished observations). An innate source of type-2 cytokines must therefore exist and, in light of the critical role played by ILCs in intestinal type-2 immunity, several recent studies have addressed potential roles for these cells in models of experimental asthma.

A number of investigators have identified type-2 ILCs in the naïve lung [23–25], consistent with their importance in initiating type-2 responses in the airways. Resembling their counterparts in the intestinal tract, these lineage-negative cells express ICOS, ST2,

CD25, and CD44, require Id2 and CD127 (IL-7R α) for their development [23, 25], and are not dependent on the transcription factor ROR γ t [25]. However, lung and intestinal type-2 ILCs are known to exhibit differences in the expression of molecules that might govern their location, such as the gut-homing chemokine receptor CCR9 (our unpublished observations). Using reporter genes for IL-4 and IL-13, a recent study demonstrated that IL-13-producing type-2 ILCs were upregulated during lung inflammation following cytokine administration (intranasal IL-25 or IL-33) or during an OVA-driven model of allergic asthma [26]. In both models, type-2 ILCs represented the major innate source of IL-13, which is known to be essential for AHR, mucus production and inflammation in these models [19, 27, 28]. Notably, adoptive transfer of IL-33-primed wild type, but not *Il13*^{-/-}, type-2 ILCs was sufficient to restore IL-25-induced AHR to otherwise resistant *Il13*^{-/-} mice. Similar results were obtained in a second study, which identified type-2 ILCs as the major source of IL-5 and IL-13 in AHR induced by the fungal aeroallergen, *Alternaria alternata* [23]. Exposure to intranasal *Alternaria* extract resulted in the rapid (<1 h) induction of IL-33 in the airways, followed several hours later by a burst of type-2 cytokines and eosinophilia. IL-5 and IL-13 induction required the IL-33 receptor subunit ST2 and originated from an innate cellular source, since the cytokine burst persisted in *Rag1*^{-/-} mice. Similar to the study by Barlow et al. [26], type-2 ILCs isolated from the lungs of naïve wild-type mice restored the *Alternaria*-induced AHR response to nonresponsive *Il7r*^{-/-} mice.

Recently, a third study has examined NKT cell-driven AHR induced by the glycolipid antigen, α -Galactosylceramide (α -GalCer) [24]. Similar to the response elicited by other intranasally administered antigens, α -GalCer drove IL-33 production, which appeared to be critical to the inflammatory response since AHR was abrogated by ST2 blockade or gene deficiency. The sources of IL-33 were identified as alveolar macrophages, dendritic cells, and type II pneumocytes, and IL-33 production was dependent upon CD1d-mediated interaction of these cells with NKT cells. The major IL-33 responsive population was type-2 ILCs, which again proved to be an important innate source of IL-13. Interestingly, however, NKT cells also produced IL-13 in this model, consistent with previous observations in IL-25-induced AHR [29], and were as efficient as type-2 ILCs in restoring AHR when adoptively transferred to *Il13*^{-/-} hosts. This raises the question as to whether type-2 ILCs represent the universal innate source of IL-13 in AHR, or whether additional innate sources could compensate for this function in their absence. The recent identification of ROR α as an important transcription factor in type-2 ILC development should now enable examination of the specific roles played by ILCs in this process. Interestingly, one study indicates that ROR α -deficient mice are partially protected from AHR [30]. While a more detailed examination is required and a role for ROR α in other cells must be investigated, it is tempting to speculate that type-2 ILCs might play a nonredundant role in the AHR response.

In addition to their essential role in providing IL-13, type-2 ILCs produce IL-5, IL-6, IL-9, and IL-10 when cultured with IL-33 [12, 23] and reporter mice have recently been used to identify ILCs that secrete IL-5 and IL-9 in vivo [31, 32]. Both studies identified

lin⁻CD90⁺CD25⁺ST2⁺ cells, which exhibit considerable overlap in their secretion of IL-5, IL-9, and IL-13, consistent with their being classified here as type-2 ILCs. Notably, a minority of cells produces IL-4 and it is possible that this source of cytokine may contribute to IL-4-dependent Th2 differentiation [12, 32]. The IL-9 fate-mapping (fm) reporter revealed that IL-9^{fm+} ILCs accumulated during papain-induced lung inflammation and were the predominant source of IL-9, as assessed by intracellular cytokine staining [32]. IL-9 production by these cells appeared to be transient and could be induced by IL-33 in vivo, and to a lesser extent IL-25. IL-9^{fm+} ILCs almost universally expressed IL-13 and production of this cytokine correlated with IL-9 availability, suggesting a potential positive feedback mechanism for cytokine secretion. Notably, production of IL-9, but not the other type-2 cytokines, required the provision of IL-2 from adaptive immune cells. This may be relevant to the broader question as to whether components of the adaptive response, most likely T cells, are required for the maintenance of ILCs during an immune response. Such an interaction seems likely, since helminth-infected *Rag2*^{-/-} mice fail to maintain type-2 ILCs over the course of infection [12] and because type-2 ILCs are maintained throughout what would typically be considered the adaptive phase of an OVA-driven AHR response [26]. The exact nature of this interplay between the innate and adaptive arms of type-2 immunity, and its physiological consequences, awaits further investigation.

Among the experimental models of allergic asthma described above, a common theme emerges in that, upon antigen challenge, type-2 ILCs represent the predominant cell type responding to the epithelial cytokines IL-33 and IL-25. Following stimulation, type-2 ILCs deliver an essential, early burst of type-2 cytokines, which serve to recruit other cellular components of type-2 immunity and amplify the inflammatory response. In addition to a key role in initiating this allergic response, it remains to be determined whether type-2 ILC-derived cytokines, particularly IL-13, are involved in maintaining inflammation and whether these factors might also play a role in driving pulmonary fibrosis, which is a significant complication of chronic inflammation [27, 28, 33].

Type-2 ILCs during influenza infection

Viral respiratory tract infections constitute a major environmental trigger for asthma exacerbations and, while the mechanisms underlying this synergism are poorly understood, a mouse model of rhinovirus infection revealed that viral infection enhanced type-2 responses to allergens, and the associated lung inflammation and AHR [34]. This exacerbation correlated with elevated levels of the type-2 cytokines IL-13 and IL-4 and intriguingly two recent studies have highlighted important roles for type-2 ILCs in experimental mouse models of influenza virus infection [25, 35]. Both groups report a lung-resident ILC population, which increases in frequency during infection and is capable of secreting the type-2 cytokine, IL-13. The specific roles identified for type-2 ILCs in these models of infection are, however, quite distinct. Chang et al. [35] report that type-2 ILCs are responsible for driving acute AHR in re-

sponse to infection, while Monticelli et al. [25] describe a role for ILCs in promoting lung repair after infection. While representing seemingly opposing roles, these two potential functions of ILCs are not necessarily mutually exclusive.

AHR in response to the influenza subtype H3N1, reported by Chang et al. [35], occurred independently of adaptive immunity and required ST2, suggesting that, similar to allergic asthma, the IL-33-ST2 signaling pathway is required for this response. Consistent with this mechanism, IL-33 was induced upon viral infection and an examination of IL-33 producing cells in the early phase of the response (24 h) suggested that alveolar macrophages might represent a major source of this cytokine. H3N1-induced AHR was critically dependent on IL-13, similar to that observed in models of allergic asthma [19] and adoptive transfer of IL-33-primed type-2 ILCs demonstrated that only those cells capable of IL-13 secretion could restore AHR to *Il13*^{-/-} hosts. Type-2 ILCs are therefore an important source of IL-13 in H3N1-induced AHR, which recapitulates many of the features of allergic asthma.

In the second study, Monticelli et al. [25] identified a contrasting role for type-2 ILCs in the restoration of lung-tissue homeostasis after influenza (H1N1) infection. Using an anti-CD90.2 antibody to deplete ILCs in *Rag1*^{-/-} mice, these investigators highlighted a role for ILCs in the maintenance of epithelial integrity and lung function, which correlated with a reduction in morbidity of H1N1-infected mice. Repair and remodeling of lung tissue could be reinstated through the adoptive transfer of naïve allotypic CD90.1⁺lin⁻ST2⁺ cells, conclusively demonstrating that type-2 ILCs are both necessary and sufficient to promote lung repair. ILC-mediated lung repair involved epithelial cell hyperplasia but was not a function of IL-13 production, since administration of recombinant IL-13 did not recapitulate this result. Similarly, the effect was not mediated by IL-22, as had been suggested by a previous study [36]. Taking a genomic approach to elucidating the mechanism of ILC action, Monticelli et al. identified a gene signature in ILCs from naïve mice that was reminiscent of the gene expression pattern identified in whole lung following LPS-induced acute lung injury. This included a number of genes associated with tissue remodeling and wound healing and included the gene encoding amphiregulin, a member of the epidermal growth factor family. Administration of amphiregulin alone was sufficient to restore lung function and epithelial cell integrity in influenza-infected mice, highlighting a key mechanism by which type-2 ILCs might promote the repair of lung tissue. The fact that the ILC gene signature resembles that induced during LPS-induced inflammation hints that ILCs might be important players in the restoration of homeostasis after acute lung injury. Consistent with this assertion, *RORα*-deficient mice, which lack functional type-2 ILCs, are more susceptible to LPS-induced lung inflammation [37].

Thus, type-2 ILCs are induced during viral infection, most likely in response to IL-33 or IL-25 produced by lung-resident cells. Depletion studies suggest these cells do not appear to play a major role in viral clearance and in fact produce no IL-17A, and little or no IFN- γ [12, 25, 35]. Instead, lung ILCs play divergent roles in promoting airway inflammation through IL-13 secretion and promoting lung-tissue repair via the production of factors such as

amphiregulin. It remains to be determined whether the same type-2 ILC fulfils each of these inflammatory and reparative roles and whether the differences in function reflect the different viruses used or the kinetics of the response. Nevertheless, it seems likely that these two aspects of type-2 ILC function may occur in parallel over the course of infection. Indeed, IL-33 induces both IL-13 and amphiregulin secretion from ILCs [25] so the effects mediated by these cytokines could manifest simultaneously (Fig. 2).

Equivalent populations in human airways

Populations equivalent to type-2 ILCs have recently been identified in humans, both in the gut and the airways. Lin⁻CD127⁺ST2⁺CRTH2⁺ cells were reported to be resident in the lung parenchyma and BAL and furthermore are enriched in nasal polyps from patients with chronic rhinosinusitis, a chronic type-2 inflammatory condition characterized by eosinophilia and high concentrations of IgE [25, 38]. These cells share a number of markers with mouse type-2 ILCs, including CD25 and ST2, and also produce IL-13 in response to the cytokines IL-25 and IL-33 [38]. Similar to their equivalents in the mouse, cells isolated from foetal gut did not produce IL-17A or IL-22 and were negative for the NKp44 receptor, thus distinguishing them from their ROR γ t-dependent, IL-22 producing counterparts [38].

Aside from their association with polyps in chronic rhinosinusitis, little is yet known about the potential involvement of type-2 ILCs in human asthma. However, components of the type-2 ILC signaling axis, namely IL-33, IL-13, and *RORα* have recently been identified by genome wide association and functional screens as being relevant to susceptibility to human asthma [39–41]. It is therefore tempting to speculate that the same pathways identified as being critical to murine type-2 ILC development and AHR may be relevant to the human airways.

An antiinflammatory role for IL-22-secreting lung ILCs

ILCs that secrete IL-22 have recently been identified in the lungs of mice during a model of experimental allergic asthma [42]. IL-22 is an IL-10 family member that may exhibit pro- or antiinflammatory functions, depending upon the inflammatory context [43]. IL-22 contributes to tissue repair and the maintenance of epithelial barriers. Consistent with these roles, the heterodimeric receptor for IL-22, comprising subunits IL-22R1 and IL-10R2, is present on nonhematopoietic cells in skin, intestine, and lung. The role of IL-22 in the airways is currently not well understood but it has been reported that, similar to IL-17A [44], IL-22 is required for asthma onset but has a protective effect during established inflammation, potentially through its effects on dendritic cells [45, 46].

In a recent report, IL-22 production was enhanced in the context of OVA-induced allergic airway inflammation and the predominant source of this cytokine was lineage⁻CD90.2⁺Sca-1⁺ innate

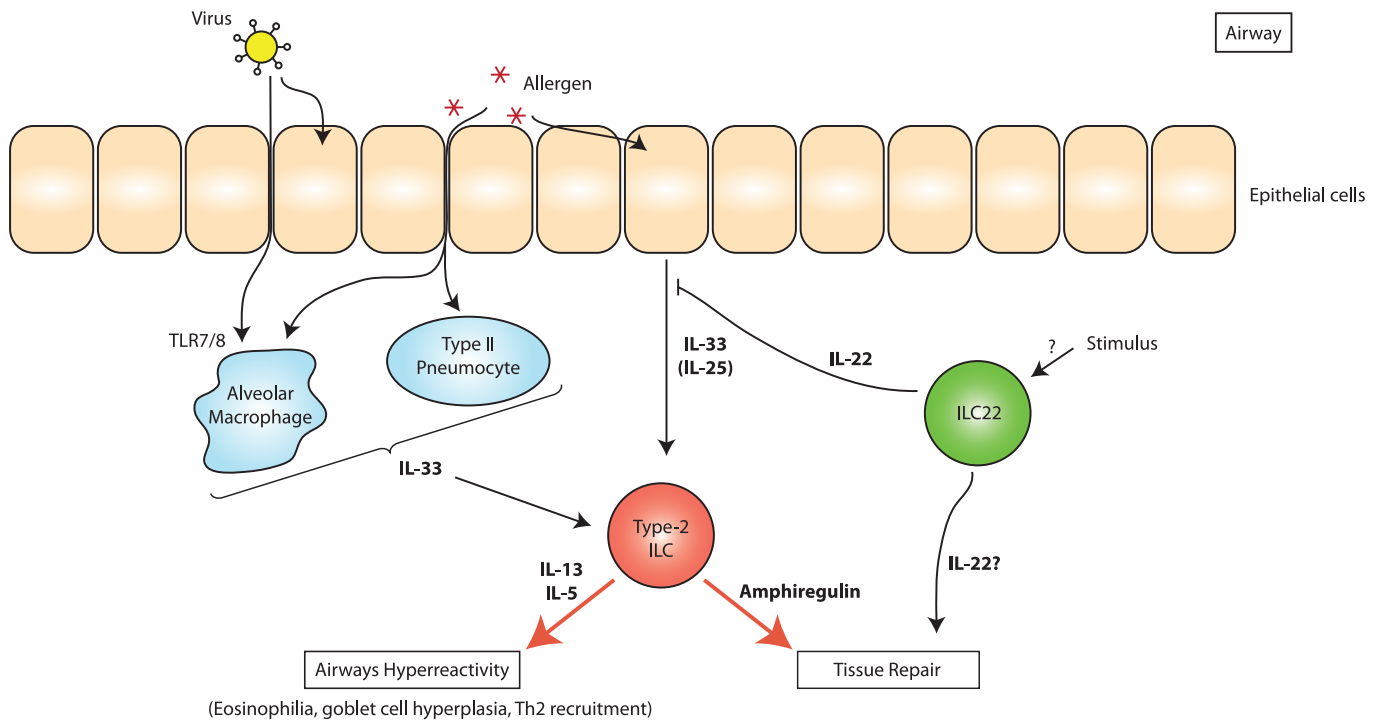


Figure 2. The role of IL-33 signaling and ILCs in AHR and lung homeostasis. Upon antigen challenge or viral infection, resident lung cells such as alveolar macrophages, type II pneumocytes and epithelial cells may be induced to secrete IL-33. This cytokine, in conjunction with other factors such as IL-25, activates type-2 ILCs to produce type-2 cytokines, such as IL-13, which drive eosinophilic lung inflammation and airway hyperreactivity. Perhaps simultaneously, IL-33 also drives type-2 ILCs to produce wound-healing factors, such as amphiregulin, which promote the repair of inflamed lung tissue. Concurrently, ILC22 cells may be recruited by as yet unknown signals and induced to secrete IL-22, which may promote lung repair directly or serve to dampen the production of cytokines from lung epithelial cells.

lymphoid cells [42]. Fate mapping experiments demonstrated that these cells had expressed *Rorc*, and a proportion also produced IL-17A, consistent with their being members of the *Rorc*/IL-22/IL-17A branch of the innate lymphoid cell family. Experiments using *Il22*^{-/-} mice demonstrated that IL-22 plays a protective role in limiting AHR and airway inflammation. Consistent with these findings, the AHR response could be exacerbated using an anti-IL-22 antibody, or ameliorated by the administration of exogenous IL-22. Similar results were obtained in a second study [47], although T cells were identified as the predominant source of IL-22 in that case.

Treatment of IL-13-stimulated murine epithelial cell lines with IL-22 reduced their production of *Il25* mRNA [47] and the chemokine CCL17 [42], which is important for the recruitment of CCR4-expressing Th2 cells. Interestingly, the abundance of IL-25 and CCL17 in BAL correlated inversely with IL-22 availability in OVA-sensitized mice [42,47] and anti-IL-25 treatment was sufficient to reverse the proinflammatory effect of anti-IL-22 [47]. Although not proving a direct mechanistic link, this evidence suggests that diminution of IL-25 production from epithelial cells might represent a means by which IL-22 could exert its anti-inflammatory effects. Interestingly, neutralization of IL-25 is protective during the AHR response, resulting in diminished levels of IL-13 and an enhancement in the production of IL-17A, which has been shown to attenuate established AHR [44, 48, 49]. IL-17A may represent an important downstream effector of this antiinflamma-

tory pathway, since IL-17A neutralization over rides the protective effect of IL-22 during AHR [46]. While the relevant cellular sources of IL-22 during airway inflammation have yet to be conclusively identified, the suppression of IL-25 secretion by epithelial cells and concomitant elevation in IL-17A may represent plausible mechanisms through which ILC22 cells could exert a protective effect during lung inflammation.

Conclusions

The contribution of the adaptive immune system to AHR has been well characterized but until recently the critical roles played by cells of the innate immune system have been underappreciated. Similar to ILCs in the intestinal tract, airway ILCs represent key mediators of lung inflammation and AHR, while also having additional roles to play in the restoration of lung tissue after inflammation. The precise signals that balance the pro- and anti-inflammatory activities of ILCs await investigation, as do the physiologically relevant sources of ILC-promoting cytokines, such as IL-33 and IL-25. It also remains to be determined how the ILCs present in the lung relate to those that reside within the intestinal tract and whether the same population of lung ILCs can bring about homeostatic and inflammatory responses. Equivalent populations of ILCs exist in the human airways and therefore represent new therapeutic targets for the prevention and management of inflammatory lung disease.

Acknowledgments: J.A.W. was supported by a grant from the American Asthma Foundation. A.N.J.M. was supported by funding from the Medical Research Council, the American Asthma Foundation, and Centocor Inc. We thank J. Barlow for critical appraisal of the manuscript.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

References

- Spits, H. and Di Santo, J. P., The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat. Immunol.* 2011. 12: 21–27.
- Vivier, E., Raulet, D. H., Moretta, A., Caligiuri, M. A., Zitvogel, L., Lanier, L. L., Yokoyama, W. M. et al., Innate or adaptive immunity? The example of natural killer cells. *Science* 2011. 331: 44–49.
- Gascoyne, D. M., Long, E., Veiga-Fernandes, H., de Boer, J., Williams, O., Seddon, B., Coles, M. et al., The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. *Nat. Immunol.* 2009. 10: 1118–1124.
- Kamizono, S., Duncan, G. S., Seidel, M. G., Morimoto, A., Hamada, K., Grosveld, G., Akashi, K. et al., Nfil3/E4bp4 is required for the development and maturation of NK cells in vivo. *J. Exp. Med.* 2009. 206: 2977–2986.
- Spits, H. and Cupedo, T., Innate lymphoid cells: emerging insights in development, lineage relationships, and function. *Annu. Rev. Immunol.* 2012. 30: 647–675.
- van de Pavert, S. A. and Mebius, R. E., New insights into the development of lymphoid tissues. *Nat. Rev. Immunol.* 2010. 10: 664–674.
- Buonocore, S., Ahern, P. P., Uhlig, H. H., Ivanov, I., Littman, D. R., Maloy, K. J. and Powrie, F., Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature* 2010. 464: 1371–1375.
- Satoh-Takayama, N., Vosschenrich, C. A., Lesjean-Pottier, S., Sawa, S., Lochner, M., Rattis, F., Mention, J. J. et al., Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. *Immunity* 2008. 29: 958–970.
- Takatori, H., Kanno, Y., Watford, W. T., Tato, C. M., Weiss, G., Ivanov, I., Littman, D. R. et al., Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. *J. Exp. Med.* 2009. 206: 35–41.
- Fallon, P. G., Ballantyne, S. J., Mangan, N. E., Barlow, J. L., Dasvarma, A., Hewett, D. R., McIlgorm, A. 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.
- Hurst, S. D., Muchamuel, T., Gorman, D. M., Gilbert, J. M., Clifford, T., Kwan, S., Menon, S. 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.
- Neill, D. R., Wong, S. H., Bellosi, A., Flynn, R. J., Daly, M., Langford, T. K., Bucks, C. et al., Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 2010. 464: 1367–1370.
- Moro, K., Yamada, T., Tanabe, M., Takeuchi, T., Ikawa, T., Kawamoto, H., Furusawa, J. et al., Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature* 2010. 463: 540–544.
- Price, A. E., Liang, H. E., Sullivan, B. M., Reinhardt, R. L., Eisle, C. J., Erle, D. J. and Locksley, R. M., Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc. Natl. Acad. Sci. USA* 2010. 107: 11489–11494.
- Brickshawana, A., Shapiro, V. S., Kita, H. and Pease, L. R., Lineage(-)Sca1+c-Kit(-)CD25+ cells are IL-33-responsive type 2 innate cells in the mouse bone marrow. *J. Immunol.* 2011. 187: 5795–5804.
- Wong, S. H., Walker, J. A., Jolin, H. E., Drynan, L. F., Hams, E., Camelo, A., Barlow, J. L. et al., Transcription factor RORalpha is critical for nuocyte development. *Nat. Immunol.* 2012. 13: 229–236.
- Kim, H. Y., DeKruyff, R. H. and Umetsu, D. T., The many paths to asthma: phenotype shaped by innate and adaptive immunity. *Nat. Immunol.* 2010. 11: 577–584.
- Voehringer, D., Reese, T. A., Huang, X., Shinkai, K. and Locksley, R. M., Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. *J. Exp. Med.* 2006. 203: 1435–1446.
- Walter, D. M., McIntire, J. J., Berry, G., McKenzie, A. N., Donaldson, D. D., DeKruyff, R. H. and Umetsu, D. T., Critical role for IL-13 in the development of allergen-induced airway hyperreactivity. *J. Immunol.* 2001. 167: 4668–4675.
- Cohn, L., Tepper, J. S. and Bottomly, K., IL-4-independent induction of airway hyperresponsiveness by Th2, but not Th1, cells. *J. Immunol.* 1998. 161: 3813–3816.
- Oliphant, C. J., Barlow, J. L. and McKenzie, A. N., Insights into the initiation of type 2 immune responses. *Immunology* 2011. 134: 378–385.
- Kondo, Y., Yoshimoto, T., Yasuda, K., Futatsugi-Yumikura, S., Morimoto, M., Hayashi, N., Hoshino, T. 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.
- Bartemes, K. R., Iijima, K., Kobayashi, T., Kephart, G. M., McKenzie, A. N. and Kita, H., IL-33-responsive lineage-CD25+CD44hi lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. *J. Immunol.* 2012. 188: 1503–1513.
- Kim, H. Y., Chang, Y. J., Subramanian, S., Lee, H. H., Albacker, L. A., Matangkasombut, P., Savage, P. B. et al., Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity. *J. Allergy Clin. Immunol.* 2012. 129: 216–227, e216.
- Monticelli, L. A., Sonnenberg, G. F., Abt, M. C., Alenghat, T., Ziegler, C. G., Doering, T. A., Angelosanto, J. M. et al., Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat. Immunol.* 2011. 12: 1045–1054.
- Barlow, J. L., Bellosi, A., Hardman, C. S., Drynan, L. F., Wong, S. H., Cruickshank, J. P. and McKenzie, A. N., Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. *J. Allergy Clin. Immunol.* 2012. 129: 191–198, e194.
- Fallon, P. G., Emson, C. L., Smith, P. and McKenzie, A. N., IL-13 overexpression predisposes to anaphylaxis following antigen sensitization. *J. Immunol.* 2001. 166: 2712–2716.
- Zhu, Z., Homer, R. J., Wang, Z., Chen, Q., Geba, G. P., Wang, J., Zhang, Y. and Elias, J. A., Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J. Clin. Invest* 1999. 103: 779–788.
- Terashima, A., Watarai, H., Inoue, S., Sekine, E., Nakagawa, R., Hase, K., Iwamura, C. et al., A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity. *J. Exp. Med.* 2008. 205: 2727–2733.

- 30 Jaradat, M., Stapleton, C., Tilley, S. L., Dixon, D., Erikson, C. J., McCaskill, J. G., Kang, H. S. et al., Modulatory role for retinoid-related orphan receptor alpha in allergen-induced lung inflammation. *Am. J. Respir. Crit. Care Med.* 2006. **174**: 1299–1309.
- 31 Ikutani, M., Yanagibashi, T., Ogasawara, M., Tsuneyama, K., Yamamoto, S., Hattori, Y., Kouro, T. et al., Identification of innate IL-5-producing cells and their role in lung eosinophil regulation and antitumor immunity. *J. Immunol.* 2012. **188**: 703–713.
- 32 Wilhelm, C., Hirota, K., Stieglitz, B., Van Snick, J., Tolaini, M., Lahl, K., Sparwasser, T. 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.
- 33 Wilson, M. S. and Wynn, T. A., Pulmonary fibrosis: pathogenesis, etiology and regulation. *Mucosal. Immunol.* 2009. **2**: 103–121.
- 34 Bartlett, N. W., Walton, R. P., Edwards, M. R., Aniscenko, J., Caramori, G., Zhu, J., Glanville, N. et al., Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat. Med.* 2008. **14**: 199–204.
- 35 Chang, Y. J., Kim, H. Y., Albacker, L. A., Baumgarth, N., McKenzie, A. N., Smith, D. E., Dekruyff, R. H. et al., Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity. *Nat. Immunol.* 2011. **12**: 631–638.
- 36 Guo, H. and Topham, D. J., Interleukin-22 (IL-22) production by pulmonary natural killer cells and the potential role of IL-22 during primary influenza virus infection. *J. Virol.* 2010. **84**: 7750–7759.
- 37 Stapleton, C. M., Jaradat, M., Dixon, D., Kang, H. S., Kim, S. C., Liao, G., Carey, M. A. et al., Enhanced susceptibility of staggerer (RORalphasg) mice to lipopolysaccharide-induced lung inflammation. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2005. **289**: L144–L152.
- 38 Mjosberg, J. M., Trifari, S., Crellin, N. K., Peters, C. P., van Drunen, C. M., Piet, B., Fokkens, W. J. et al., Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CCR2 and CD161. *Nat. Immunol.* 2011. **12**: 1055–1062.
- 39 Gudbjartsson, D. F., Bjornsdottir, U. S., Halapi, E., Helgadottir, A., Sulem, P., Jonsdottir, G. M., Thorleifsson, G. et al., Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat. Genet.* 2009. **41**: 342–347.
- 40 Moffatt, M. F., Gut, I. G., Demenais, F., Strachan, D. P., Bouzigon, E., Heath, S., von Mutius, E. et al., A large-scale, consortium-based genomewide association study of asthma. *N. Engl. J. Med.* 2010. **363**: 1211–1221.
- 41 Prefontaine, D., Nadigel, J., Chouiali, F., Audusseau, S., Semlali, A., Chakir, J., Martin, J. G. et al., Increased IL-33 expression by epithelial cells in bronchial asthma. *J. Allergy Clin. Immunol.* 2010. **125**: 752–754.
- 42 Taube, C., Tertilt, C., Gyulveszi, G., Dehzad, N., Kreymborg, K., Schneeweiss, K., Michel, E. et al., IL-22 is produced by innate lymphoid cells and limits inflammation in allergic airway disease. *PLoS One* 2011. **6**: e21799.
- 43 Zenewicz, L. A. and Flavell, R. A., Recent advances in IL-22 biology. *Int. Immunol.* 2011. **23**: 159–163.
- 44 Schnyder-Candrian, S., Togbe, D., Couillin, I., Mercier, I., Brombacher, F., Quesniaux, V., Fossiez, F. et al., Interleukin-17 is a negative regulator of established allergic asthma. *J. Exp. Med.* 2006. **203**: 2715–2725.
- 45 Schnyder, B., Lima, C. and Schnyder-Candrian, S., Interleukin-22 is a negative regulator of the allergic response. *Cytokine* 2010. **50**: 220–227.
- 46 Besnard, A. G., Sabat, R., Dumoutier, L., Renaud, J. C., Willart, M., Lambrecht, B., Teixeira, M. M. et al., Dual role of IL-22 in allergic airway inflammation and its cross-talk with IL-17A. *Am. J. Respir. Crit. Care Med.* 2011. **183**: 1153–1163.
- 47 Takahashi, K., Hirose, K., Kawashima, S., Niwa, Y., Wakashin, H., Iwata, A., Tokoyoda, K. et al., IL-22 attenuates IL-25 production by lung epithelial cells and inhibits antigen-induced eosinophilic airway inflammation. *J. Allergy Clin. Immunol.* 2011. **128**: 1067–1076, e1061–e1066.
- 48 Barlow, J. L., Flynn, R. J., Ballantyne, S. J. and McKenzie, A. N., Reciprocal expression of IL-25 and IL-17A is important for allergic airways hyperactivity. *Clin. Exp. Allergy* 2011. **41**: 1447–1455.
- 49 Ballantyne, S. J., Barlow, J. L., Jolin, H. E., Nath, P., Williams, A. S., Chung, K. F., Sturton, G. et al., Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma. *J. Allergy Clin. Immunol.* 2007. **120**: 1324–1331.

Abbreviations: ILC: innate lymphoid cell · LTi: lymphoid tissue inducer

Full correspondence: Dr. Andrew McKenzie, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, United Kingdom.
 Fax: +44-0-1223-412178
 e-mail: anm@mrc-lmb.cam.ac.uk

Received: 30/1/2012
 Revised: 16/3/2012
 Accepted: 11/4/2012