Published in final edited form as:
J Allergy Clin Immunol. 2012 January ; 129(1): 216–227.e6. doi:10.1016/j.jaci.2011.10.036.

Innate lymphoid cells responding to IL-33 mediate airway-hyperreactivity independent of adaptive immunity

Hye Young Kim, PhD[a,*], **Ya-Jen Chang, PhD**[a,*], **Srividya Subramanian, PhD**[a], **Hyun-Hee Lee, PhD**[a], **Lee A. Albacker, PhD**[a], **Ponpan Matangkasombut, MD,PhD**[a], **Paul B. Savage, PhD**[b], **Andrew N. J. McKenzie, PhD**[c], **Dirk E. Smith, PhD**[d], **James B. Rottman, PhD**[e], **Rosemarie H. DeKruyff, PhD**[a], and **Dale T. Umetsu, MD,PhD**[a]

[a]Division of Immunology and Allergy, Children's Hospital, Harvard Medical School, Boston, MA, USA

[b]Brigham Young University, Provo, Utah, USA

[c]MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB20QH, UK

[d]Department of Inflammation Research, Amgen, Seattle, WA, USA

[e]Department of Pathology, Amgen, Cambridge, MA, USA

Abstract

Background—Asthma has been considered an immunological disease mediated by Th2 cells and adaptive immunity. However, clinical and experimental observations suggest that additional pathways may regulate asthma, particularly in non-allergic forms of asthma, such as asthma associated with air pollution, stress, obesity and infection.

Objectives—Our goal was to understand Th2 cell-independent conditions which might lead to airway hyperreactivity (AHR), a cardinal feature of asthma.

Methods—We examined a mouse model of experimental asthma, in which AHR was induced with glycolipid antigens, which activate natural killer T (NKT) cells.

Results—In this model, AHR developed rapidly when mice were treated with NKT cell-activating glycolipid antigens, even in the absence of conventional $CD4^+$ T cells. The activated NKT cells directly induced alveolar macrophages to produce IL-33, which in turn activated NKT cells as well as natural helper cells, a newly described non-T, non-B, innate lymphoid cell type, to increase production of IL-13. Surprisingly, this glycolipid-induced AHR pathway required not only IL-13, but also IL-33 and its receptor, ST2, since it was blocked by an anti-ST2 mAb, and was greatly reduced in $ST2^{-/-}$ mice. When adoptively transferred into $IL\text{-}13^{-/-}$ mice, both wildtype natural helper cells and NKT cells were sufficient for the development of glycolipid induced AHR.

Address correspondence to: Dr. Dale T. Umetsu, Division of Immunology, Karp Laboratories, Rm 10127, Children's Hospital, Harvard Medical School, One Blackfan Circle, Boston, MA 02115, USA. Phone: 617.919.2439; Fax:617.730.0384; dale.umetsu@childrens.harvard.edu.
*These individuals contributed equally to the completion of these studies.

Conflict of interest: Dirk E. Smith and James B. Rottman are employees and shareholders of Amgen Corporation. The other authors have declared that no conflicts of interest exist.

Conclusion—Since plant pollens, house dust and some bacteria contain glycolipids that can directly activate NKT cells, these studies suggest that AHR and asthma can fully develop, or be greatly enhanced, through innate immune mechanisms, involving IL-33, natural helper cells and NKT cells.

Keywords

Innate; NKT; Natural Helper cells; T_H2; IL-33; IL-13; glycolipid; asthma

Introduction

Asthma, which has increased dramatically in prevalence over the past 2-3 decades, is a major public health problem that affects 300 million individuals worldwide [1]. Although allergic inflammation guided by $CD4^+$ Th2 cells and eosinophils is thought to play a dominant role in the pathogenesis of asthma, several clinical and experimental observations suggest that additional pathological mechanisms may underlie the development of at least some forms of asthma [2]. For example, non-allergic asthma, triggered by environmental factors, such as air pollutants (e.g., smoke, diesel particles, and ozone), stress, obesity and infection, appear to develop independently of Th2 cells [3-7]. In addition, non-Th2 factors such as IFN-γ, IL-17 and neutrophils are frequently found in the lungs of patients with asthma, particularly in the lungs of patients with severe asthma or of patients with corticosteroid resistant asthma. Moreover, Th2-targeted therapies, including anti-IL-4 mAb, anti-IL-5 mAb and IL-13 antagonists, have not been uniformly effective as hoped in many clinical trials of asthma [8]. These findings suggest that asthma is heterogeneous, and that other cell types and pathways that include innate immune cells and their associated cytokines, may also regulate the development of asthma [9].

To further understand the conditions under which airway hyperreactivity (AHR), a cardinal feature of asthma, might occur in the absence of Th2 cells and adaptive immunity, we examined a model of experimental asthma, in which natural killer T (NKT) cells activated with glycolipid antigens, induced AHR [10]. In systems where AHR is induced with allergen, the presence of both IL-13-producing Th2 cells and NKT cells is required [11]. In contrast, when induced directly by glycolipid-activated NKT cells, AHR occurred rapidly in the absence of Th2 cells and adaptive immunity, for example in MHC class $II^{-/-}$ mice treated with NKT cell-activating glycolipid antigens, including glycolipid antigens isolated from bacteria. We now show surprisingly, that glycolipid antigen-induced AHR required IL-33 and its receptor, ST2, since it was blocked by an anti-ST2 mAb, and was greatly reduced in $ST2^{-/-}$ mice. Importantly, in the lungs, a newly described non-T, non-B, innate lymphoid cell type called natural helper cells or nuocytes responded to IL-33, produced by alveolar macrophages and dendritic cells that directly interacted with NKT cells. Both natural helper cells and NKT cells responded to IL-33 by producing significant quantities of IL-13, which induced AHR [12, 13]. When adoptively transferred to $IL\text{-}13^{-/-}$ mice, both wildtype NKT cells and natural helper cells were sufficient for the development of glycolipid induced AHR. Since plant pollens [14], house dust [15] and some bacteria, including bacteria found in the lungs of patients with poorly controlled asthma [16-18], contain glycolipids that can directly activate NKT cells, we suggest that AHR and airway inflammation can develop in the complete absence of Th2 cells, or be greatly enhanced, through innate immune mechanisms, involving IL-33, natural helper cells and NKT cells.

Material and Methods

Mice

Wild-type BALB/c ByJ, Rag2$^{-/-}$ mice were generated by Dr. Shizuo Akira, and B6.129-H2 dlAb1-Ea/J (MHC II$^{-/-}$ mice) on the C57BL/6 background were purchased from The Jackson Laboratory (Bar Harbor, Maine). CD1d$^{-/-}$ and Jα18$^{-/-}$ mice were gifts from Michael Grusby (Harvard School of Public Health) and Masaru Taniguchi/Toshinori Nakayama (Chiba University) respectively. ST2$^{-/-}$ and IL4$^{-/-}$/IL-13$^{-/-}$ mice were generated by Andrew McKenzie (Oxford, UK). Female mice were studied at 6~8 wks of age and were age matched. The Animal Care and Use Committee, Children's Hospital Boston approved all animal protocols.

Antibodies and Reagents

α-GalactosylCeramide (α-GalCer), *Sphingomonas* glycolipid (PS-30), and its corresponding vehicle control were synthesized by Paul B. Savage (Brigham Young University, Provo Utah). Recombinant human IL-33, anti-mouse ST2 blocking Ab and the isotype control rat IgG1$_k$ Ab were generated at Amgen (Thousand Oaks, CA).

Measurement of AHR

Mice were anesthetized with pentobarbitol (7.5-10mg/mice) and AHR was assessed by invasive measurement of airway resistance, in which anesthetized and tracheostomized mice were mechanically ventilated modified version of a described method (Buxco Electronics)[11].

ELISA

Total lung homogenate or cell culture supernatants were collected and measured by IL-33 ELISA kit (ebioscience).

Statistical tests

Data are given as mean ± SEM, and were analyzed by ANOVA or unpaired student's *t*-tests (two-tailed) (Prism 4; GraphPad Software Inc., San Diego, CA). $p<0.05$(*), $p<0.01$ (**) and $p<0.001$ (***).

Results

α-GalCer induced AHR is IL-33 dependent

Administration of α-GalCer to wild-type BALB/c mice rapidly (within 24 hrs) induced a robust AHR response associated with significant airway inflammation. The rapid development (within 24 hrs) of α-GalCer-induced AHR suggested that innate rather than adaptive mechanisms were involved. We therefore assessed the role of an IL-33 - ST2 (IL-33 receptor) axis, and showed that AHR and airway inflammation induced by α-GalCer was blocked by treatment with an ST2 blocking mAb (Fig. 1A, 1B and 1C). These results contrast with that of allergen-induced AHR, which occurs independently of ST2 and IL-33 [19, 20]. The requirement for IL-33 and ST2 in α-GalCer-induced AHR was confirmed by challenging ST2$^{-/-}$ mice with α-GalCer, which resulted in a reduced AHR and airway inflammatory response, compared to that observed in wild-type mice (Fig.1D, E). Histological evaluation of the lungs confirmed the significant reduction in airway inflammation in the ST2$^{-/-}$ mice treated with α-GalCer compared to wild-type BALB/c mice (Fig. 1F), indicating that the induction of AHR by α-GalCer occurred in an ST2 dependent, and presumably an IL-33 dependent, manner.

α-GalCer increases production of IL-33 in the lung

The development of α-GalCer induced AHR was associated with a rapid increase in IL-33 in extracts of the lung, in terms of mRNA (Fig. 2A) and protein (Fig. 2B). Assessment of IL-33 by immunohistochemistry indicated that IL-33 was produced by alveolar macrophages and type II pneumocytes (identified as surfactant protein C^+) after α-GalCer stimulation (Fig. 2C, D). Furthermore, IL-33 immunoreactivity was weakly detected in airway epithelial cells, and may have increased after α-GalCer stimulation (Fig. 2C). To confirm the apparent IL-33 expression in these populations, we assessed IL-33 by intracellular cytokine staining of dispersed lung cells. IL-33 was found in alveolar macrophages ($F4/80^+CD11c^+$), interstital macrophages ($F4/80^+$ $CD11c^-$) [21] and DCs ($F4/80^-CD11c^+$), and the number of these cells greatly increased after challenge with α-GalCer (Fig. 2E and 2F). Taken together, these results indicated that IL-33 production in the lungs by alveolar macrophages, DCs and type II pneumocytes significantly increased after α-GalCer challenge. Note that IL-33 expression by type II pneumocytes was nuclear in location, as has been previously described, possibly functioning as a transcriptional repressor [22, 23].

NKT cells, but not Th2 cells induce IL-33 in macrophages and DCs

To determine the mechanism by which α-GalCer induced IL-33 production in the antigen presenting cells, we co-cultured NKT cells with macrophages, DCs, an airway epithelial cell line (MLE12) and a Type II pneumocyte line (A549). Co-culture of NKT cells with alveolar macrophages or $CD11c^+$ lung DCs greatly increased IL-33 mRNA levels, compared to macrophages or DCs cultured alone, and this was further increased by the addition of α-GalCer (Fig. 3A, B). Culture of NKT cells with the airway epithelial cell line, MLE, did not increase IL-33 mRNA (Fig. 3C), but culture of human NKT cells with a Type II pneumocyte epithelial cell line, A549, which expresses CD1d (Supl Fig. 1), greatly increased IL-33 mRNA (Fig. 3D). The induction of IL-33 mRNA by NKT cells in alveolar macrophages and DCs was specific and CD1d dependent, since it was blocked by anti-CD1d mAb (HB323) (Fig 3A, B and D), but not by anti-CD40L or anti-ST2 mAb(Fig 3E, F). The response of the alveolar macrophages cultured with NKT cells in the absence of α-GalCer was most likely due to the presence of an endogenous glycolipid present in the antigen presenting cell. The dependence on CD1d was further demonstrated by co-culture of the NKT cells with alveolar macrophages or lung DC from $CD1d^{-/-}$ mice, which failed to produce IL-33 protein (Fig. 3G, H). The secretion of IL-33 protein required direct contact between the NKT cell and alveolar macrophage (Fig. 3I and Supl Fig. 2A), and was partially reduced by blockade of macrophage apoptosis (Fig. 3J). The induction of IL-33 required NKT cells and not Th2 cells, since OVA-specific Th2 cells (generated from T cells from DO11.10 transgenic mice) co-cultured with alveolar macrophages, DCs or airway epithelial cell lines and OVA, caused only minimal induction of IL-33 mRNA in these cells (Supl Fig. 2B-D). These results suggest that NKT cells activated by α-GalCer, but not OVA-activated Th2 cells, induced alveolar macrophages, DCs and type II pneumocytes to express increased amounts of IL-33.

Sphingomonas glycolipid also induces AHR in ST2 dependent manner

The list of glycolipid antigens recognized by NKT cells is growing rapidly, and includes several endogenous glycolipids [24], glycolipids found in pollen [14], house dust [15], as well as in a number of bacteria [16, 17]. Fig. 4A shows that one glycolipid from a *Sphingomonas* species, PS30, which is known to activate NKT cells, induced significant AHR and airway inflammation in wild-type BALB/c mice, but not in $ST2^{-/-}$ mice (Fig. 4A, 4B), indicating a requirement for the IL-33/ST2 axis. The importance of PS30 is highlighted by recent studies showing that bacteria in the Sphingomonadaceae family, which include *Sphingomonas* species, are commonly found in the lungs of patients with poorly controlled asthma, and are associated with the presence of AHR [18]. Moreover, administration of PS30 to mice increased IL-33 expression by $CD11c^+$ DCs and $F4/80^+$ macrophages (Fig. 4C), and

increased production of IL-33 in the lung (Fig. 4D). In addition, PS30 activated NKT cells, which increased expression of CD25 and CD69 (Fig. 4E). These data suggest that glycolipids from bacteria can activate NKT cells to induce AHR, in an IL-33/ST2 dependent fashion.

α-GalCer induced AHR is associated with an increase in natural helper cells

Since IL-13 is required for α-GalCer induced AHR [12], we asked which cell types were required. Following the administration of α-GalCer, we found that a large fraction of non-T non-B innate cells produced the bulk of IL-13 (Fig. 5A). Recently, a novel innate lymphoid cell type called natural helper cells, nuocytes, multipotent progenitor cells or innate Type 2 cells, has been identified that responds to IL-33 by producing large quantities of IL-13 [25-28]. These innate lymphocytes are present in fat-associated lymphoid clusters [25] and in mesenteric lymph nodes of helminth-infected mice [26, 27], and in the lungs of influenza A virus infected mice [7]. Surprisingly, we found natural helper cells/nuocytes in the lung, identified as lineage$^-$ (CD3, CD19, FcεRIα, CD11b, CD11c and CD49b), ST2$^+$ and c-Kit$^+$. Flow cytometric analysis showed that 1-2% of the total the lung cells from naïve mice was natural helper cells (Fig. 5B). After α-GalCer treatment, the total numbers of these ST2$^+$ c-Kit$^+$ natural helper cells expressing Sca-1 and producing IL-13 greatly increased although the percentage of Lin$^-$ST2$^+$ cells were slightly reduced (Fig. 5C). In addition, PS30, a glycolipid from *Sphingomonas* also increased the number of Sca1$^+$ natural helper cells in the lung (Supl. Fig. 3). Moreover, treatment of ST2$^{-/-}$ mice with α-GalCer failed to increase IL-13 production in lung natural helper cells (Fig. 5D). Taken together, these results suggest that α-GalCer or *Sphingomonas* glycolipid treatment induced AHR by activating NKT cells, which induced macrophages, DCs and Type II pneumocytes to produce IL-33, which in turn activated natural helper cells to produce IL-13, a known inducer of AHR [12, 13].

IL-33 induces AHR in the absence of adaptive immunity

Previous studies have reported that intra-nasal administration of IL-33 induces AHR and airway inflammation in RAG$^{-/-}$ mice [29], but the specific cells responding to the IL-33 was not clear, particularly since IL-33 was not required for allergen-induced AHR [7, 19, 20], although the resolution of AHR occurred more rapidly when IL-33 was neutralized [30]. We confirmed that administration of IL-33 to RAG2$^{-/-}$ mice for 3 consecutive days resulted in the induction of AHR and airway inflammation without adaptive immune cells (Supl. Fig 4). Importantly, administration of IL-33 greatly increased the number of natural helper cells in the lungs of both wildtype and RAG2$^{-/-}$ mice (Fig. 6A). The natural helper cells produced large quantities of IL-13 (Fig. 6B) as well as IL-5 (data not shown) in response to IL-33. IL-13 production by the natural helper cells was required for IL-33 induced AHR, as challenge of IL-13$^{-/-}$ mice with IL-33 failed to induce AHR (Fig. 6C), and airway inflammation (Fig. 6D). IL-5 production by natural helper cells did not result in airway eosinophilia, most likely because IL-33 induces epithelial cells to produce CXCL1(KC)/IL-8 [31, 32], a chemokine that attracts neutrophils, and because IL-33 directly activates neutrophils. Although IL-33 can activate mast cells to produce IL-4 and IL-13, which might induce AHR, administration of IL-33 to mast cell deficient mice still resulted in AHR, suggesting that mast cells are not the primary target cell of IL-33 in asthma [29]. These results taken together indicate that activation of natural helper cells producing IL-13 by IL-33 is sufficient for the induction of AHR in absence of adaptive immunity.

Adoptive transfer of IL-13 producing natural helper cells or NKT cells

We assessed the role of natural helper cells or NKT cells in this model with reconstitution experiments (Fig. 7A). Adoptive transfer of a very limited number of lung natural helper cells (10^4/mouse) from wild-type mice into IL-13$^{-/-}$ mice fully reconstituted α-GalCer induced AHR and airway inflammation, where as adoptive transfer of lung natural helper

cells without α-GalCer stimulation failed to reconstitute AHR (Fig. 7B,C). These data suggested that the IL-13 producing natural helper cells were sufficient for α-GalCer induced AHR. In addition, the role of IL-13 producing NKT cells was confirmed by a separate adoptive transfer experiment, showing that adoptive transfer of purified wild-type NKT cells also reconstituted α-GalCer induced AHR and airway inflammation (Fig. 7C, D). Furthermore, ST2 expression on pulmonary NKT cells or primary NKT cell lines was increased after intranasal challenge with α-GalCer (Supl. Fig. 5A,5B), and stimulation of the NKT cells with both α-GalCer and IL-33 synergistically enhanced cytokine production and expression of activation markers from NKT cells (Supl. Fig. 5C, 5D). Therefore, these results suggest that α-GalCer stimulation increased NKT cell responsiveness to IL-33. Further, these results suggest that either IL-13 producing natural helper cells or NKT cells are sufficient for the development of α-GalCer-induced AHR, and that IL-33 plays an important role in the development of innate forms of asthma.

Discussion

In these studies, we found that innate lymphoid cells including natural helper cells and NKT cells, utilizing an IL-33/ST2 receptor axis, could induce the development of AHR in the absence of Th2 cells and adaptive immunity. In this pathway, activation of NKT cells resulted in the induction of IL-33 production in alveolar macrophages and DCs, which then drove the subsequent activation and expansion of natural helper cells and/or NKT cells producing IL-13 and the development of AHR (Fig. 8). Because NKT cells can be directly activated by glycolipids from pollens and house dust [15, 33], this pathway may greatly enhance the development of allergen-induced asthma. Moreover, this innate pathway may be very important in patients with non-allergic forms of asthma, since this IL-33-mediated pathway could develop in the absence of Th2 cells and adaptive immunity, and because NKT cells can be activated by glycolipids expressed during viral infection [6], and ozone exposure [4], by pollen, or by bacteria [16, 34-36], including *Sphingomonas* species, which are found in the lungs of patients with poorly controlled asthma and associated with AHR [18]. Therefore, these studies help to explain the heterogeneity in asthma, which appears to develop through several distinct pathogenic pathways, some involving allergic/adaptive mechanisms, while other involving non-allergic/innate mechanisms [2].

For example, although Th2 cells have been shown to drive the development of AHR in experimental models of allergic asthma, in another model of asthma, Sendai virus infection precipitated chronic lung disease associated with AHR, in the absence of Th2 cells [6]. This pathway involved alternatively activated alveolar macrophages interacting with NKT cells [6, 37], although the role of natural helper cells was not evaluated. Exposure to air pollution may result in another Th2 cell-independent pathway to asthma, which can be modeled by exposing mice repeatedly to ozone, a major component of air pollution, resulting in severe AHR associated with airway neutrophils rather than eosinophils [4]. This AHR response required NKT cells producing IL-17, but not adaptive immunity. Our current studies indicate that an additional innate pathway, involving NKT cell-driven IL-33 production by alveolar macrophages and dendritic cells can lead to the development of experimental asthma in the absence of adaptive immunity.

IL-33 is a member of the IL-1 family, and has been shown to play an important role in mediating an inflammatory response required for parasite expulsion [38], and in several autoimmune and inflammatory disorders [39, 40]. While studies of intestinal parasites suggest an important role of IL-33 in diseases associated with Th2 cytokines, the precise role of IL-33 in the lungs and in asthma has not been fully investigated. IL-33 is present in the lungs of patients with severe asthma [41], is present in the blood of patients undergoing anaphylaxis [42], and can activate DCs to prime T cells to produce Th2 cytokines [43]. In

addition, *IL33* has been identified in genome wide association studies in humans as a susceptibility gene for asthma [44, 45]. However, the conditions that result in IL-33 secretion in the lungs have not been clear, although IL-33 has been shown to be produced by airway epithelial cells activated by TLR4 ligation by dust mite allergen [46], or in the context of influenza A virus infection [7]. Our studies now indicate that IL-33 can be produced by alveolar macrophages in the lungs after direct activation by NKT cells.

Although administration of recombinant IL-33 was previously shown to induce the development of AHR even in the absence of Th2 cells (e.g., in $Rag^{-/-}$ mice) [29, 47], the cell type in these models responding to IL-33 and required for AHR was not clear, although in retrospect may have been natural helper cells. IL-33 binds to its receptor ST2, which is expressed by Th2 cells [48], eosinophils [49], mast cells, basophils [50], by some NKT cells [51], as well as by natural helper cells (nuocytes or multipotent progenitor cells) [25-27]. Natural helper cells have only recently been described and have not been appreciated in the past [25-27], and the precise characteristics of natural helper cells are still being delineated. Natural helper cells do not express lineage (Lin) markers, but express c-Kit, IL-7R, and with activation, Sca-1, and may be related to another innate non-T, non-B lymphoid cell type that is Lin^{-} c-Kit^{-}, RORγt^{+}, IL-23 $receptor^{+}$, $IL\text{-}17^{+}$ and involved in the development of colitis in mice [52] and in humans [53]. Natural helper cells described in mice may be similar to a non-T, non-B cell population found in the sputum of patients with asthma [54], and recently in human nasal polyp tissue [55]. We now show that natural helper cells are present in the lungs of mice, and in combination with NKT cells and alveolar macrophages play an overlooked role in the development of AHR. Although IL-33 can affect several cell types that contribute to asthma (eosinophils, basophils, macrophages and Th2 cells), we demonstrated that IL-33 induced IL-13 production in natural helper cells and in NKT cells, which then mediated the development of glycolipid-induced AHR.

The identification of a role for natural helper cells in AHR extends the range of cell types that can mediate AHR and asthma, perhaps reflecting the heterogeneous nature of asthma, which likely develops through several distinct pathways. Th2 cells, which also produce IL-13 and IL-5, are present in the lungs of many patients with asthma, and are essential in allergic asthma. Thus, in experimental models, Th2 cells responding to allergen are required for the induction of allergen-induced AHR, usually in combination with NKT cells [11, 56], although Th2 cells, when adoptively transferred in large numbers are capable by themselves of inducing AHR [57]. NKT cells are also essential for the induction of AHR in some models, independent of Th2 cells, as in ozone induced AHR by producing IL-17 [4] and in Sendai virus induced AHR by producing IL-13 [6]. We now show that natural helper cells producing IL-13 are also sufficient for inducing the development of AHR, independent of Th2 cells, but in partnership with NKT cells. Natural helper cells, when activated through a pathway involving influenza A virus, may also induce AHR independent of Th2 cells or NKT cells [7]. Although each of these pathways may mediate a distinct form of asthma and may occur independently of each other, it is possible that in some patients, these distinct pathways may coexist, for example in patients with multiple triggers for asthma. In such patients, these separate pathways may synergize, and result in more severe disease.

In summary, we identified an immunological pathway for AHR that occurs independently of Th2 cells, but which is dependent on NKT cells and natural helper cells. This pathway is also dependent on the IL-33 receptor ST2, and on IL-33 produced by alveolar macrophages, dendritic cells and Type II pneumocytes. We suggest that some environmental agents, such as bacteria, pollens and house dust antigen, which can directly activate NKT cells, may trigger the development of AHR and asthma through such an innate pathway. Since this innate pathway may be relatively resistant to current treatments for asthma (e.g.,

corticosteroids), a greater understanding of NKT cells, natural helper cells and IL-33 in this pathway could lead to improved therapies for this heterogeneous disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

AHR	airway hyperreactivity
NKT	Natural Killer T
DC	Dendtritic cell
α-GalCer	α-GalactosylCeramide
WT	wild-type

References

1. GINA. Global strategy for asthma management and prevention. 2010. www.ginasthma.org
2. Kim HY, DeKruyff RH, Umetsu DT. The many paths to asthma: phenotype shaped by innate and adaptive immunity. Nat Immunol. 2010; 11:577–84. [PubMed: 20562844]
3. Johnston RA, Zhu M, Rivera-Sanchez YM, Lu FL, Theman TA, Flynt L, et al. Allergic airway responses in obese mice. Am J Respir Crit Care Med. 2007; 176:650–8. [PubMed: 17641156]
4. Pichavant M, Goya S, Meyer EH, Johnston RA, Kim HY, Matangkasombut P, 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; 205:385–93. [PubMed: 18250191]
5. Li N, Hao M, Phalen RF, Hinds WC, Nel AE. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. Clin Immunol. 2003; 109:250–65. [PubMed: 14697739]
6. Kim EY, Battaile JT, Patel AC, You Y, Agapov E, Grayson MH, et al. Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease. Nat Med. 2008; 14:633–40. [PubMed: 18488036]
7. Chang Y-J, Kim H, Albacker L, Baumgarth N, McKenzie A, Smith D, et al. Innate lymphoid cells mediate influenza-induced airway hyperreactivity independent of adaptive immunity. Nature Immunol. 2011; 12:631–8. [PubMed: 21623379]
8. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet. 2008; 372:1107–19. [PubMed: 18805339]
9. Matangkasombut P, Pichavant M, Dekruyff RH, Umetsu DT. Natural killer T cells and the regulation of asthma. Mucosal Immunol. 2009; 2:383–92. [PubMed: 19587638]
10. Meyer EH, Goya S, Akbari O, Berry GJ, Savage PB, Kronenberg M, 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 U S A. 2006; 103:2782–7. [PubMed: 16478801]
11. Akbari O, Stock P, Meyer E, Kronenberg M, Sidobre S, Nakayama T, et al. Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. Nature Medicine. 2003; 9:582–88.
12. Wills-Karp M, Luyimbazi J, Xu X, Schofield b, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. Science. 1998; 282:2258–61. [PubMed: 9856949]
13. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. Science. 1998; 282:2261–3. [PubMed: 9856950]
14. Agea E, Russano A, Bistoni O, Mannucci R, Nicoletti I, Corazzi L, et al. Human CD1-restricted T cell recognition of lipids from pollens. J Exp Med. 2005; 202:295–308. [PubMed: 16009719]

15. Wingender G, Rogers P, Batzer G, Lee MS, Bai D, Pei B, et al. Invariant NKT cells are required for airway inflammation induced by environmental antigens. J Exp Med. 2011
16. Mattner J, Debord KL, Ismail N, Goff RD, Cantu C 3rd, Zhou D, et al. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature. 2005; 434:525–9. [PubMed: 15791258]
17. Kinjo Y, Wu D, Kim G, Xing GW, Poles MA, Ho DD, et al. Recognition of bacterial glycosphingolipids by natural killer T cells. Nature. 2005; 434:520–5. [PubMed: 15791257]
18. Huang YJ, Nelson CE, Brodie EL, Desantis TZ, Baek MS, Liu J, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. J Allergy Clin Immunol. 2011; 127:372–81. e1-3. [PubMed: 21194740]
19. Hoshino K, Kashiwamura S, Kuribayashi K, Kodama T, Tsujimura T, Nakanishi K, et al. The absence of interleukin 1 receptor-related T1/ST2 does not affect T helper cell type 2 development and its effector function. J Exp Med. 1999; 190:1541–8. [PubMed: 10562328]
20. Mangan NE, Dasvarma A, McKenzie AN, Fallon PG. T1/ST2 expression on Th2 cells negatively regulates allergic pulmonary inflammation. Eur J Immunol. 2007; 37:1302–12. [PubMed: 17407196]
21. Bedoret D, Wallemacq H, Marichal T, Desmet C, Quesada Calvo F, Henry E, et al. Lung interstitial macrophages alter dendritic cell functions to prevent airway allergy in mice. J Clin Invest. 2009; 119:3723–38. [PubMed: 19907079]
22. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, et al. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. Proc Natl Acad Sci U S A. 2007; 104:282–7. [PubMed: 17185418]
23. Kouzaki H, Iijima K, Kobayashi T, O'Grady SM, Kita H. The danger signal, extracellular ATP, is a sensor for an airborne allergen and triggers IL-33 release and innate Th2-type responses. J Immunol. 2011; 186:4375–87. [PubMed: 21357533]
24. Zhou D, Mattner J, Cantu C 3rd, Schrantz N, Yin N, Gao Y, et al. Lysosomal glycosphingolipid recognition by NKT cells. Science. 2004; 306:1786–9. [PubMed: 15539565]
25. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. Nature. 2010; 463:540–4. [PubMed: 20023630]
26. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature. 2010; 464:1367–70. [PubMed: 20200518]
27. Saenz SA, Siracusa MC, Perrigoue JG, Spencer SP, Urban JF Jr, Tocker JE, et al. IL25 elicits a multipotent progenitor cell population that promotes T(H)2 cytokine responses. Nature. 2010; 464:1371–6. [PubMed: 20393462]
28. Price AE, Liang HE, Sullivan BM, Reinhardt RL, Eisley CJ, Erle DJ, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. Proc Natl Acad Sci U S A. 2010; 107:11489–94. [PubMed: 20534524]
29. Kondo Y, Yoshimoto T, Yasuda K, Futatsugi-Yumikura S, Morimoto M, Hayashi N, 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. [PubMed: 18448455]
30. Kearley J, Barker JE, Robinson DS, Lloyd CM. Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4+CD25+ regulatory T cells is interleukin 10 dependent. J Exp Med. 2005; 202:1539–47. [PubMed: 16314435]
31. Verri WA Jr. Souto FO, Vieira SM, Almeida SC, Fukada SY, Xu D, et al. IL-33 induces neutrophil migration in rheumatoid arthritis and is a target of anti-TNF therapy. Ann Rheum Dis. 2010; 69:1697–703. [PubMed: 20472598]
32. Yagami A, Orihara K, Morita H, Futamura K, Hashimoto N, Matsumoto K, et al. IL-33 mediates inflammatory responses in human lung tissue cells. J Immunol. 2010; 185:5743–50. [PubMed: 20926795]
33. Agea E, Russano A, Bistoni O, Mannucci R, Nicoletti I, Corazzi L, et al. Human CD1-restricted T cell recognition of lipids from pollens. J Exp Med. 2005; 202:295–308. [PubMed: 16009719]

34. Kinjo Y, Wu D, Kim G, Xing G-W, Poles M, Ho DD, et al. Recognition of bacterial glycosphingolipids by natural killer T cells. Nature. 2005; 434:520–5. [PubMed: 15791257]
35. Kinjo Y, Tupin E, Wu D, Fujio M, Garcia-Navarro R, Benhnia MR, et al. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. Nat Immunol. 2006; 7:978–86. [PubMed: 16921381]
36. Kim S, Lalani S, Parekh VV, Vincent TL, Wu L, Van Kaer L. Impact of bacteria on the phenotype, functions, and therapeutic activities of invariant NKT cells in mice. J Clin Invest. 2008; 118:2301–15. [PubMed: 18451996]
37. Subrata LS, Bizzintino J, Mamessier E, Bosco A, McKenna KL, Wikstrom ME, et al. Interactions between innate antiviral and atopic immunoinflammatory pathways precipitate and sustain asthma exacerbations in children. J Immunol. 2009; 183:2793–800. [PubMed: 19620293]
38. Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. J Exp Med. 2000; 191:1069–76. [PubMed: 10727469]
39. Mu R, Huang HQ, Li YH, Li C, Ye H, Li ZG. Elevated Serum Interleukin 33 Is Associated with Autoantibody Production in Patients with Rheumatoid Arthritis. J Rheumatol. 2010
40. Pastorelli L, Garg RR, Hoang SB, Spina L, Mattioli B, Scarpa M, et al. Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis. Proc Natl Acad Sci U S A. 2010; 107:8017–22. [PubMed: 20385815]
41. Prefontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. J Immunol. 2009; 183:5094–103. [PubMed: 19801525]
42. Pushparaj PN, Tay HK, H'Ng SC, Pitman N, Xu D, McKenzie A, et al. The cytokine interleukin-33 mediates anaphylactic shock. Proc Natl Acad Sci U S A. 2009; 106:9773–8. [PubMed: 19506243]
43. Besnard AG, Togbe D, Guillou N, Erard F, Quesniaux V, Ryffel B. IL-33-activated dendritic cells are critical for allergic airway inflammation. Eur J Immunol. 2011; 41:1675–86. [PubMed: 21469105]
44. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med. 2010; 363:1211–21. [PubMed: 20860503]
45. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet. 2009; 41:342–7. [PubMed: 19198610]
46. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. Nat Med. 2009; 15:410–6. [PubMed: 19330007]
47. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, 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; 23:479–90. [PubMed: 16286016]
48. Lohning M, Hutloff A, Kallinich T, Mages HW, Bonhagen K, Radbruch A, et al. Expression of ICOS in vivo defines CD4+ effector T cells with high inflammatory potential and a strong bias for secretion of interleukin 10. J Exp Med. 2003; 197:181–93. [PubMed: 12538658]
49. Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. J Allergy Clin Immunol. 2008; 121:1484–90. [PubMed: 18539196]
50. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. Nat Rev Immunol. 2010; 10:103–10. [PubMed: 20081870]
51. Smithgall MD, Comeau MR, Yoon BR, Kaufman D, Armitage R, Smith DE. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. Int Immunol. 2008; 20:1019–30. [PubMed: 18550585]
52. Buonocore S, Ahern PP, Uhlig HH, Ivanov II, Littman DR, Maloy KJ, et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. Nature. 2010; 464:1371–5. [PubMed: 20393462]

53. Geremia A, Arancibia-Carcamo CV, Fleming MP, Rust N, Singh B, Mortensen NJ, et al. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. J Exp Med. 2011

54. Allakhverdi Z, Comeau MR, Smith DE, Toy D, Endam LM, Desrosiers M, et al. CD34+ hemopoietic progenitor cells are potent effectors of allergic inflammation. J Allergy Clin Immunol. 2009; 123:472–8. [PubMed: 19064280]

55. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. Nat Immunol. 2011

56. Lisbonne M, Diem S, de Castro Keller A, Lefort J, Araujo L, Hachem P, et al. Cutting edge: invariant V alpha 14 NKT cells are required for allergen-induced airway inflammation and hyperreactivity in an experimental asthma model. J Immunol. 2003; 171:1637–41. [PubMed: 12902459]

57. Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. J. Clin. Invest. 1999; 103:175–83. [PubMed: 9916129]

Key messages

- This study examines a novel pathway for the development of experimental asthma that occurs in the absence of Th2 cells and adaptive immunity.
- This pathway for airway hyperreactivity (AHR), a cardinal feature of asthma, is initiated by the activation of NKT cells, which induce alveolar macrophages to produce IL-33, which in turn stimulates NKT cells and natural helper cells, a newly described effector cell type, to increase production of IL-13, a cytokine critical for the development of AHR.
- Since allergens and bacteria in the lungs of asthmatics contain glycolipids that can activate NKT cells, these studies provide a mechanism by which NKT cells, natural helper cells, and IL-33 drive the development of AHR.

Capsule summary

In an experimental mouse model, asthma developed through a pathway involving IL-33-producing alveolar macrophages, natural helper cells and NKT cells. These results may explain non-allergic forms of asthma that develop independently of Th2 cells.

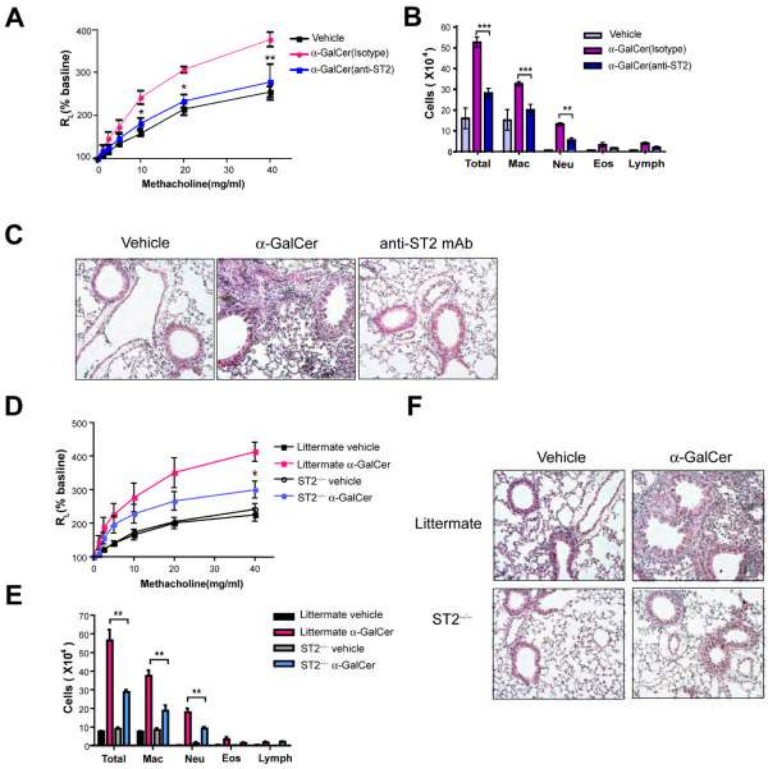

Figure 1. Blockade of IL-33 receptor, ST2, abrogates α-GalCer induced AHR

(A) Anti-mouse ST2 blocking Ab or rat $IgG1_k$ isotype control Ab were given intravenously to the mice 24 hrs before intranasal administration of 0.5μg α-GalCer or vehicle. This data shows the mean ± SEM % of saline value and representative of three experiments. α-GalCer + anti-ST2 mAb treated group was compared with α-GalCer + isotype control mAb treated group. $p<0.05$ (*) and $p<0.01$ (**).

(B) Data represent the number of cells per ml in BAL fluid. Mac, macrophage; Neu, neutrophils; Eos, eosinophils; Lymph, lymphocytes. $p<0.01$ (**) and $p<0.001$ (***).

(C) 24h after intranasal α-GalCer challenge, lung tissues from each group were sectioned, and stained with hematoxylin/eosin (X40)

(D) Littermate control and $ST2^{-/-}$ mice were challenged with α-GalCer or vehicle, and AHR was determined by invasive measurement of airway resistance (R_L) as in (A). $p<0.05$ (*)

(E) Number of cells in BAL fluid was counted as in (B). Mac, macrophage; Neu, neutrophils; Eos, eosinophils; Lymph, lymphocytes. $p<0.05$ (*) and $p<0.01$ (**).

(F) Lung tissues from each group were stained with hematoxylin/eosin as Fig. 1C. Data are representative of at least three experiments.

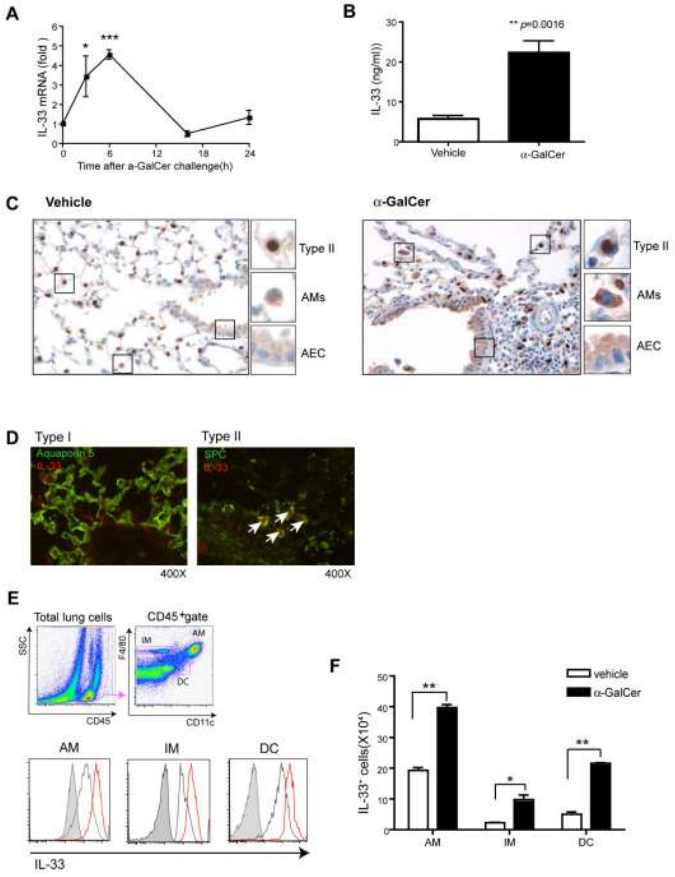

Figure 2. αGalCer induces AHR by enhancing IL-33 production in the lung

(A) IL-33 mRNA was normalized to GAPDH and relative mRNA levels calculated as fold increase over vehicle control. $p<0.05$ (*) and $p<0.001$ (***).

(B) Total lung homogenates were taken from vehicle or α-GalCer treated mice and IL-33 protein levels were tested by ELISA. $p<0.01$ (**).

(C) Lung tissues from vehicle treated mice (left) or α-GalCer treated mice (right) were evaluated by IHC for IL-33 expression and immunolocalization. The higher power images on the right of each panel show type II pneumocytes, alveolar macrophages (AM), and airway epithelial cell (AEC). Original magnification: X200.

(D) IL-33 expression by Type I pnemocytes (left: Aquaporin 5 positive) or Type II pnemocytes (right: surfactant protein C positive) were identified using lung tissues. The arrows indicate IL-33$^+$ cells, which are surfactant protein C positive (type II pneumocytes).

(E) IL-33 expression was examined by intracellular cytokine staining. Shaded: isotype control, black line: vehicle, and red line; αGalCer treatment.

(F) The graph represents the total numbers of IL-33 producing cells in the lung. $p<0.05$ (*) and $p<0.01$ (**). (n≥9)

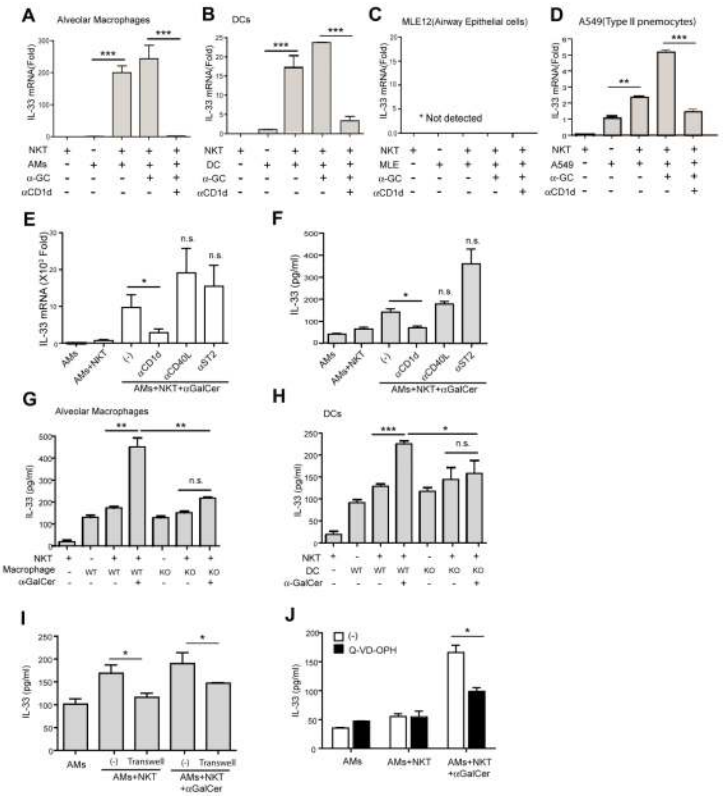

Figure 3. NKT cells induce IL-33 production from alveolar macrophages, DCs and Type II penumocytes

(A-C) Mouse NKT cells line were co-cultured with alveolar macrophages (A), DCs (B), or mouse airway epithelial cell lines (MLE12) (C). Relative mRNA levels calculated as fold increase over alveolar macrophages (A), or DCs (B). For CD1d blocking, alveolar macrophages or DCs treated with 10μg/ml of anti-CD1d mAb (HB323) 1hr before co-culture. Data present the mean ± SEM, representative of three experiments. $p<0.001$ (***)

(D) Human NKT cells (10^5/96 well) were co-cultured with human type II pneumocytes epithelial cell lines (A549) (5×10^4/96 well). 10μg/ml of anti-CD1d mAb (42.1) was used for CD1d blocking experiments. Relative mRNA levels calculated as fold increase over Type II pnemocytes. $p<0.05$ (*) and $p<0.001$ (***).

(E, F) The interaction between NKT cells and alveolar macrophages was blocked by anti-CD1d mAb, anti-CD40L mAb, and anti-ST2 mAb (10μg/ml). IL-33 expression was measured by mRNA expression (E) or ELISA (F). $p<0.05$ (*).

(G, H) Alveolar macrophages (G) or DCs (H) from WT or $CD1d^{-/-}$ mice co-cultured with NKT cells for 72 hrs. $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

(I) NKT cells were placed in upper chamber and alveolar macrophages were placed in lower chamber of transwell chambers to block the NKT cells-Macrophage cell-cell contact. $p<0.05$ (*).

(J) To inhibit the apoptosis of macrophage, 10 μg/ml of apoptosis inhibitor (Q-VD-OPH) was treated to macrophages. $p<0.05$ (*). Data are representative of at least three experiments.

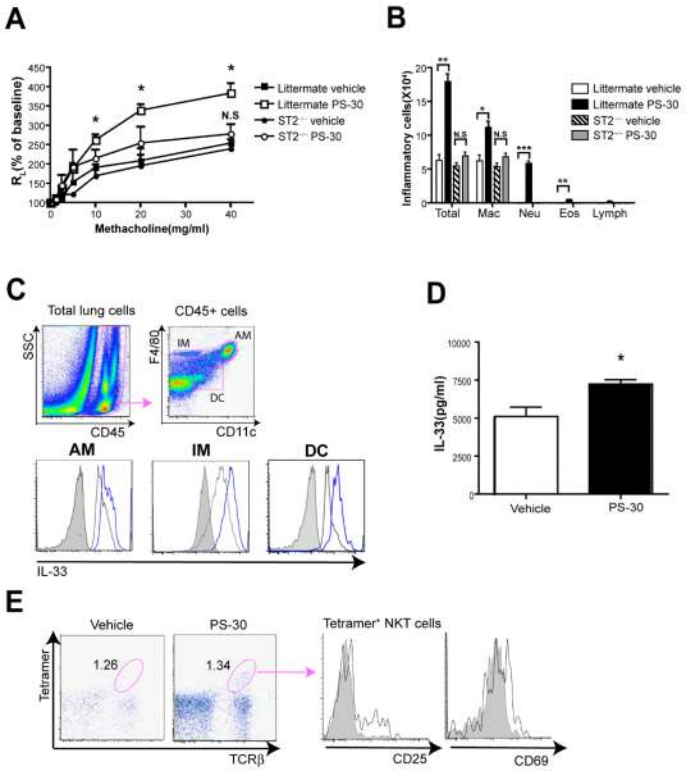

Figure 4. Glycolipid from *Sphingomonas* induces AHR by producing IL-33

(A) 10μg of *Sphingomonas* glycolipid (PS-30) was administrated to littermate control or ST2$^{-/-}$ mice. 24hr after glycolipid challenge, AHR was measured as in Fig. 1A. (n≥6) p<0.05 (*).

(B) Data represent the number of cells per ml in BAL fluid and are the mean ± SEM. p<0.05 (*), p<0.01 (**) and p<0.001 (***). Mac, macrophage; Neu, neutrophils; Eos, eosinophils; Lymph, lymphocytes.

(C) Lung cells were stained as in Fig 2 (D) 24hr after *Sphingomonas* glycolipid(PS-30) challenge. Shaded histogram, isotype control; black line, vehicle; blue line, PS-30 treated group.

(D) IL-33 protein levels from lungs of mice at 24 hrs after treatment with vehicle or PS-30, measured by ELISA. p<0.05 (*).

(E) BAL fluid was collected 24hr after PS-30 treatment, and NKT cells were analyzed Shaded histogram, vehicle control; black line, PS-30 exposed NKT cells. The MFI for CD25 was 147 (control, 94.7), and for CD69:1236 (control,885) Data are representative of at least three experiments (n≥6).

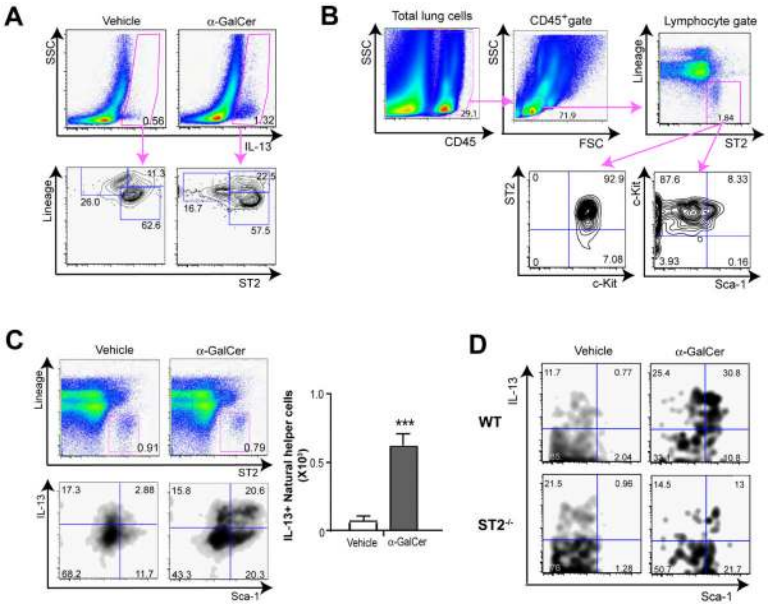

Figure 5. α-GalCer induces IL-13 producing natural helper cells in the lung

(A) Total lung cells were obtained from vehicle or α-GalCer treated mice, and level of IL-13 on CD45 positive cells detected by intracellular staining (upper panel). Total IL-13 positive cells were further analyzed using Abs against Lin and ST2 (lower panel).

(B) Natural helper cells ($lin^{-}ST2^{+}$) subsets were gated from CD45 positive cells, and then assessed by the expression of c-Kit and Sca-1.

(C) Natural helper cells ($CD45^{+}lin^{-}ST2^{+}$) were gated as shown in (B), and the expression of Sca-1 and IL-13 were further analyzed. The graph represents the number of Lin^{-} $ST2^{+}$ c-Kit^{+} $Sca\text{-}1^{+}IL\text{-}13^{+}$ natural helper cells in the lung (MFI for vehicle: 78.6±19.1, MFI for α-GalCer: 641±128.5). $p<0.001$(***). Data are representative of at least three experiments.

(D) IL-13 producing natural helper cells from WT or $ST2^{-/-}$ mice were compared as in Fig 5C. n≥4 mice in each group in this experiment.

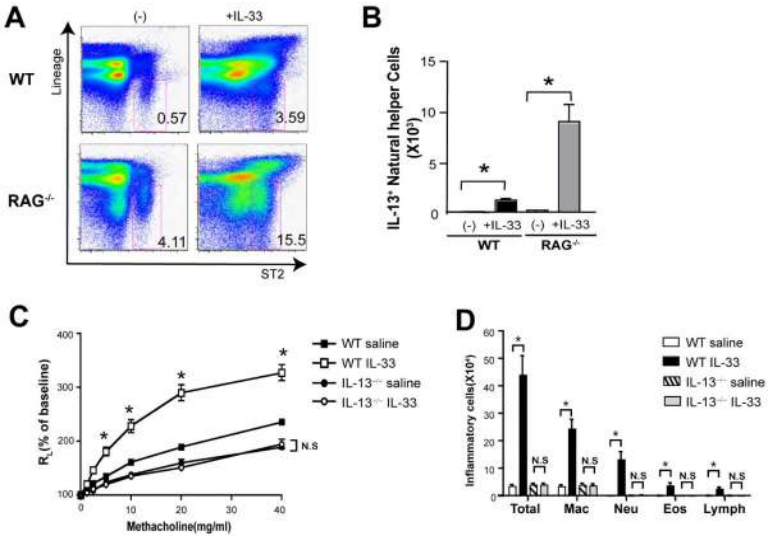

Figure 6. IL-33 induces AHR by increasing IL-13 producing natural helper cells

(A) Recombinant IL-33 (0.1μg) administrated into WT or $RAG^{-/-}$ mice for 3 consecutive days. The number of natural helper cells ($CD45^{+}lin^{-}ST2^{+}$) was assessed by flow cytometry.

(B) The number of IL-13 producing natural helper cells (Lin^{-} $ST2^{+}$ c-Kit^{+} $Sca\text{-}1^{+}IL\text{-}13^{+}$) was calculated after saline or IL-33 challenge. (WT saline: $69.1\pm11.5\times10^{3}$, WT IL-33: 1265 ± 248.7 $X10^{3}$, $RAG^{-/-}$ saline:$138.6\pm25.4\times10^{3}$ and $RAG^{-/-}$ IL-33:$927.2\pm196.8\times10^{3}$). $p<0.05$ (*).

(C) WT BALB/c mice or $IL\text{-}13^{-/-}$ mice were treated with 0.1μg of IL-33 for 3 consecutive days, and AHR was measured. Data represent the mean ± SEM % of saline value (n≥4). $p<0.05$ (*).

(D) BAL fluid from mice in (C) was analyzed for airway inflammatory cells as shown in (1B). Data represent the number of cells (mean ± SEM), representative of three experiments. $p<0.05$ (*). Mac, macrophage; Neu, neutrophils; Eos, eosinophils; Lymph, lymphocytes.

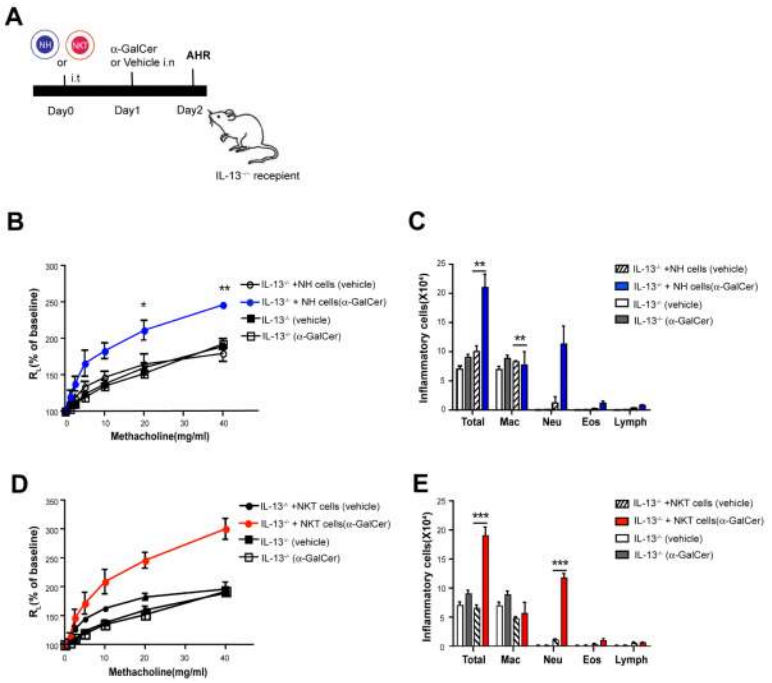

Figure 7. Natural helper cells and NKT cells are required for α-GalCer induced AHR

(A) Schematic showing the protocol for adoptive transfer of natural helper cells or NKT cells. Change in lung resistance (B) and inflammatory cells in BAL fluid (C) in IL-13$^{-/-}$ recipients (n = 4 per group) given purified natural helper cells (Lin^{-}ST2^{+} subsets) from WT donors followed by vehicle or α-GalCer challenge for 24 hr. Mac, macrophage; Neu, neutrophils; Eos, eosinophils; Lymph, lymphocytes.

Change in lung resistance (D) and inflammatory cells in BAL fluid (E) in IL-13$^{-/-}$ recipients (n = 4 per group) given purified NKT cells from WT donors followed by vehicle or α-GalCer challenge for 24 hr. Mac, macrophage; Neu, neutrophils; Eos, eosinophils; Lymph, lymphocytes.

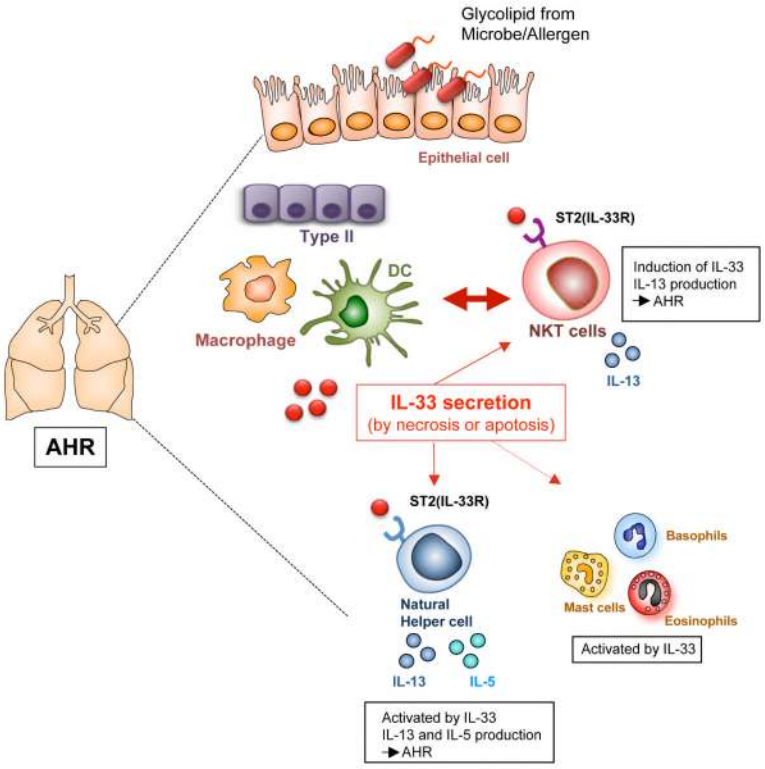

Figure 8. Schematic of the IL-33 – ST2 axis in the development of AHR

Upon activation by glycolipid antigens, NKT cells induce macrophages, DCs and Type II pneumocytes to produce IL-33, which in turn activates natural helper cells and NKT cells to produce IL-13, resulting in the development of AHR. IL-33 can also activate mast cells, eosinophils and basophils.