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Current Perspectives: focused commentary: Key cells in asthma

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Abstract

The pathogenesis of bronchial asthma, a complex trait associated with a number of environmental factors (e.g., allergens, infection, air pollution, exercise and obesity), involves multiple cell types and several distinct cellular and molecular pathways. These pathways include adaptive and innate immunity, and involve Th2 cells, mast cells, basophils, eosinophils, neutrophils, airway epithelial cells and subsets of a newly described cell type called natural killer T (NKT) cells. A role for subsets of NKT cells in asthma has been suggested by extensive studies in animal models of asthma, induced with allergen, viral infection, ozone exposure or bacterial components, suggesting that NKT cells function in concert with Th2 cells or independently of adaptive immunity in causing airway hyperreactivity. The clinical relevance of NKT cells in human asthma is supported by the observation that NKT cells are present in the lungs of some patients with asthma, particularly patients with severe poorly controlled asthma, although additional research is required to more precisely define the specific role of NKT cells in human asthma. These studies of NKT cells greatly expand our understanding of possible mechanisms that drive the development of asthma, particularly in the case of asthma associated with neutrophils, viral infection and air pollution.

Keywords

asthma; airway hyperreactivity; natural killer T cells; Th2 cells; innate immunity

Introduction

The simple clinical definition of bronchial asthma (wheezing, shortness of breath and reversible airway obstruction associated with airway hyperreactivity (AHR)) suggests that a single pathophysiological mechanism might explain the development of asthma. However, asthma is associated with numerous environmental factors including allergens, viral infection, air pollution, obesity, aspirin, acetaminophen and exercise, as well as with a host of susceptibility genes, suggesting that asthma is in fact complex, with several distinct forms that might be associated with several different pathogenic mechanisms.

Currently, the most popular paradigm regarding asthma pathogenesis involves allergen-specific Th2 cells and adaptive immunity. Allergen-specific Th2 cells are thought to be present in the lungs of virtually all patients with asthma (1), particularly in patients with

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allergic asthma, the most common form of asthma, and to mediate allergy, a major risk factor for asthma. Th2 cells orchestrate the inflammation in asthma by producing IL-4, IL-5 and IL-13, which increase airway mucus production, increase the growth and differentiation of airway eosinophils, basophils, mast cells, B cells producing IgE and Th2 cells, and directly induce the development of AHR, a cardinal feature of asthma. The Th2 paradigm has been extremely appealing in its simplicity, and has dominated the field of asthma and allergy for more than 20 years, since Mosmann and Coffman first described Th1 and Th2 cells in 1986 (2).

While the Th2 paradigm of asthma explains many features of asthma, a number of clinical observations cannot be explained by this paradigm. For example, many patients have a nonallergic form of asthma, do not respond to allergens, and have no allergen-specific Th2 cells. Furthermore, Th2 cell-independent factors, such as viruses, air pollution and exercise cause asthma symptoms in virtually all asthma patients whether or not allergy is present. In addition, other 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, and patients with steroid non-responsive asthma. Additionally, most patients who are sensitized to allergens, i.e., patients with allergic rhinitis, do not develop asthma, suggesting that Th2 cells by themselves are not sufficient for the development of asthma. Finally, Th2 targeted treatments, for example with anti-IL-4, anti-IL-5 and IL-13 antagonists have not been as effective as hoped in many clinical studies of asthma (3–5). These observations suggest that several additional processes and pathways, beyond, or in addition to, Th2 cells, must regulate the development of asthma.

NKT cells regulate immunity

One factor that could explain many of the features and contradictions in asthma is a recently described cell type, called natural killer T (NKT) cells, which were first suggested to play an important pathogenic role in asthma in 2003 (6,7). NKT cells comprise a small population of lymphocytes that express features of NK cells and conventional T cells. Most NKT cells also express a lineage specific transcription factor, PLZF (8), and an invariant T cell receptor (TCR), called V α 14 in mice and V α 24 in humans (9). This invariant TCR of NKT cells recognizes glycolipid rather than peptide antigens, in the context of the class I-like molecule, CD1d. This TCR is highly conserved in most mammalian species, suggesting that it is a pattern recognition receptor, and that NKT cells have evolved to play an important role in innate immunity. Furthermore, the activation of NKT cells through this conserved TCR results in the very rapid production of cytokines, including IFN- γ and IL-4, which activate dendritic cells, macrophages, NK cells, T cells and B cells, and drives the development of adaptive immunity. Moreover, the rapid production of cytokines by NKT cells has been shown to regulate the development of cancer, autoimmunity, ulcerative colitis, infectious diseases and asthma (9). The many distinct functions of NKT cells may be explained by the existence of several different subsets of NKT cells, determined by their expression of CD4, by their cytokine profiles, and expression of the invariant TCR or of other non-invariant TCRs (see below).

NKT cells in mouse models of asthma

Several different laboratories using several different mouse models of asthma, have reported that the presence of NKT cells is required for the development of AHR. First, in allergen-induced AHR, a model of allergic asthma, AHR failed to occur in NKT cell deficient mice (J α 18^{-/-} mice and CD1d^{-/-} mice), although some degree of eosinophilic airway inflammation remained. AHR was restored in the NKT cell deficient mice by the adoptive transfer of NKT cells from wildtype but not from IL-4/IL-13 deficient mice (6).

Furthermore, in ozone-induced AHR, a model of asthma associated with air pollution, AHR also failed to develop in NKT cell deficient mice (10). In this model with ozone, which is a major component of air pollution, AHR was associated with the presence of airway neutrophils, but not eosinophils, and with the presence of IL-17, which is a potent neutrophil chemotactic factor. Finally, in virus-induced AHR, a model of virus induced asthma, AHR also failed to develop in NKT cell deficient mice (11). In this virus model, infection with Sendai (parainfluenza) virus resulted in the development of a chronic airway inflammation, associated with AHR and increased mucus production in wildtype but not in NKT cell deficient mice.

In each of these different models of asthma, a distinct subset of NKT cells was required: in allergen-induced AHR, CD4⁺, IL-17RB⁺ (receptor for IL-25), IL-4/IL-13 producing NKT cells were required (6,12); in ozone-induced AHR, CD4⁻ (double negative, DN), NK1.1⁻, IL-17 producing NKT cells were required (10); and in Sendai virus induced AHR, CD4⁻ (DN), IL-13 producing NKT cells were required (11) (see Table 1). Moreover, both ozone-induced and virus-induced AHR could occur in the absence of adaptive immunity, in class II^{-/-} mice (which have NKT cells but not conventional CD4⁺ T cells). These results indicate that AHR can be induced by innate immune mechanisms in the absence of Th2 cells, and that NKT cell-mediated mechanisms might explain the development of several different forms of asthma, including allergic asthma, as well as asthma associated with air pollution, neutrophils or with viral infection.

AHR in $\beta 2$ microglobulin deficient mice

The requirement for NKT cells in three distinct models of AHR, suggests that NKT cells might play an important role in asthma. However, studies performed more than 10 years ago showed that $\beta 2$ microglobulin deficient ($\beta 2m^{-/-}$) mice develop severe allergen-induced AHR, suggesting that AHR might not require NKT cells (13,14), and that NKT cells might not be important in asthma (15). Until recently, the $\beta 2m^{-/-}$ mice were believed to lack expression of CD1d, since CD1d is a heterodimeric molecule that includes $\beta 2m$, and to lack CD1d restricted NKT cells. However, more recent studies indicate that CD1d can in fact be expressed in the absence of $\beta 2m$ (16–18), and that a subset of NKT cells is indeed present in $\beta 2m^{-/-}$ mice (18–20). Moreover, allergen-induced AHR that occurred in the $\beta 2m^{-/-}$ mice was abolished by treatment with several different anti-CD1d mAbs, and by an anti-NK1.1 monoclonal antibody (mAb) (depletes NK and NKT cells), but not with anti-asialo GM1 Ab (depletes only NK cells and not NKT cells) (20). Furthermore, allergen-induced AHR was restored in CD1d^{-/-} mice by adoptive transfer of an NKT-cell-enriched population from the $\beta 2m^{-/-}$ mice. The NKT cells in $\beta 2m^{-/-}$ mice do not express the invariant TCR of NKT cells, and are therefore distinct from the subset that induces AHR in wild type mice. Nevertheless, these studies together strongly suggest that the development of AHR in the $\beta 2m^{-/-}$ mice is mediated by CD1d restricted NKT cells (20), and that subsets of NKT cells are indeed required for the development of AHR in many distinct models of asthma (Table 1).

The many pathways to asthma

Are NKT cells required for the development of most forms of AHR? This question has been difficult to answer, because the concept that NKT cells are important in asthma is relatively new, and the precise mechanisms by which NKT cells induce AHR are only now beginning to be elucidated. One possible mechanism by which NKT cells function in asthma is that some subsets of NKT cells synergize with Th2 cells in allergen-induced AHR, possibly by licensing Th2 cells to induce AHR (6). Th2 cells, but not NKT cells, can clearly respond to protein allergens and induce a degree of eosinophilic inflammation. However, airway

inflammation by itself is not sufficient for asthma, and the frequency of allergen-specific Th2 cells activated by a given allergen may be too low to induce AHR without amplification by NKT cells. This might explain why mice deficient in NKT cells cannot develop allergen-induced AHR even though they clearly generate allergen-specific Th2 responses. On the other hand, when unusually large numbers of Th2 cells are present in the lungs, for example when large numbers of allergen-specific Th2 cells are transferred into SCID or RAG1^{-/-} mice, AHR can be induced by the Th2 cells in the absence of NKT cells (21). This would suggest that Th2 cells and IL-4/IL-13 producing NKT cells may have overlapping and synergistic functions.

The importance of NKT cells however, may be more apparent in situations where the Th2 paradigm fails to explain asthma. For example, AHR can develop in the absence of adaptive immunity, in association with air pollution or with viral infection. These events might be mediated by NKT cells producing IL-17 (10), or NKT cells that activate alveolar macrophages to produce IL-13 (11), respectively. During viral infection, one alternative mechanism for NKT cells in asthma pathogenesis involves the interaction between NKT cells and alternatively activated alveolar macrophages. In this model, viral infection induces IL-13 production by NKT cells, which interact with lung macrophages, resulting in significant IL-13 production by alternatively activated macrophages (11). Viral infections are particularly important in causing severe asthma exacerbations, and recent analysis of the blood from children with asthma during acute viral infection demonstrated a notable increase in CD1d⁺ IL-13R⁺ alternatively activated macrophages (22), consistent with this mouse model of viral induced AHR (11).

In several other situations where AHR may develop in the absence of adaptive immunity, environmental agents that enter the lung may directly activate NKT cells to induce AHR. For example, lipids from pollens (e.g., cypress tree pollen) can directly activate NKT cells (23), and might participate in the development of asthma, or at least in enhancing the development of pollen specific Th2 cells. Glycolipids from microorganisms that enter the lungs may also directly activate NKT cells and induce AHR, since we know that glycolipids from *Borrelia burgdorferi*, *Sphingomonas paucimobilis*, and other bacteria (24–26) can activate NKT cells through direct or indirect pathways. The development of AHR in the absence of adaptive immunity may not be so surprising, since airway inflammation and AHR can develop in the absence of T cells, for example by the administration of recombinant cytokines, such as rIL-13 (27), or rIL-33 (28), although in this latter case, the effect may be mediated by IL-13 produced by mast cells or basophils activated by IL-33. Therefore, there may be many pathways that lead to the development of AHR, some that require adaptive immunity and some that entirely bypass T cells but require innate immunity, including subsets of NKT cells.

Does an important role for NKT cells invalidate the Th2 paradigm of asthma? Absolutely not, since Th2 cells are necessary for responses to allergen and for the development of allergen-induced AHR. It is counterproductive to argue whether Th2 cells or NKT cells are more important, since both adaptive and innate immunity are important in asthma and likely interact, and since many cell types are required for the development of asthma, including Th2 cells, NKT cells, mast cells, basophils, eosinophils, neutrophils, epithelial cells and smooth muscle cells. Declaring that one cell type is more important than another fails to appreciate the complexity of asthma, and the fact that many cellular and molecular pathways are involved in the development of asthma.

NKT cells in human asthma

Although the studies of NKT cells in mouse models asthma have been compelling, and although other studies in mice were essential in predicting the importance of other cell types in human asthma, including Th2 cells, regulatory T cells, Th17 cells and dendritic cells, the clinical relevance of NKT cells in human asthma must of course be established by study of patients with asthma. To date, at least 14 studies have been performed examining bronchoalveolar (BAL) fluid and/or endobronchial biopsies from patients with asthma for the presence of NKT cells (29). While most of these studies (10 reports) found that NKT cells were increased in number in the lungs of patients with asthma, four of these studies concluded that NKT cells were not increased (30–33), resulting in some disagreement regarding the importance of NKT cells in asthma. It is possible that technical difficulties in identifying NKT cells, use of sputum rather than BAL fluid samples, lack of appropriate control populations in some studies, and most importantly, differences in the patient populations examined, particularly in terms of asthma severity, may have contributed to the discrepancies observed. This last point may be particularly important, since a more recent study of patients with a very broad range of asthma severity and symptom control, showed that the number of NKT cells present in the lung of patients with asthma varied a great deal, particularly with asthma severity and asthma control (34). Patients with severe, poorly controlled asthma had a consistent and very significant increase in the number of BAL fluid NKT cells, compared to non-asthmatic controls, whereas only about 50% of patients with well-controlled (moderate to severe) asthma had detectable increases in the number of BAL fluid NKT cells compared to controls. Therefore, the number of pulmonary NKT cells is quite variable, and many, though not all, patients with asthma have an increase in the number of pulmonary NKT cells. In contrast, normal individuals consistently have undetectable levels of pulmonary NKT cells.

The absence of detectable levels of NKT cells in the lungs of some asthma patients (particularly patients with mild or well-controlled asthma) cannot be interpreted as an indication that NKT cells are not important in asthma. It is clear that the importance of any given cell type is not determined by counting the number of cells present, but rather by defining its function. Therefore, the fact that NKT cells are present in the lungs of some but not all patients with asthma suggests that the role of NKT cells in asthma must be assessed by measuring NKT cell function, as has been done in functional studies in mice. For example, allergen or ozone challenge of wildtype but not NKT cell deficient mice increased the number of NKT cells present in the lungs, associated with an increase in AHR. Similarly, allergen challenge of patients with moderate to severe allergic asthma resulted in a significant increase in the number of NKT cells present in endobronchial biopsy specimens at 24 hrs, compared to baseline, and was associated with a significant increase in AHR (35). Although it was not possible in this study to determine whether the AHR response required the presence of NKT cells, these results confirmed a clear increase in the number of pulmonary NKT cells after allergen challenge in the asthma patients (36), and a clear absence of NKT cells in biopsies from normal individuals, all consistent with allergen challenge studies in mice showing that the development of AHR required the presence of NKT cells.

Other functional studies in mice that remain to be replicated in humans have shown that the direct activation of even the few NKT cells in the lungs of naïve mice, for example with the glycolipid α -galactosylceramide (α -GalCer) resulted in severe AHR, indicating an important role for NKT cells in mediating AHR (25). Similar functional studies in humans have not yet been performed, mainly for safety reasons. However, direct activation of pulmonary NKT cells in nonhuman primates (cynomolgus monkeys) with α -GalCer resulted in the development of significant AHR (37). Since monkeys are closely related to humans in terms

of their genome, respiratory physiology and immunological responses, these results predict that human NKT cells play an important functional role in asthma. Future functional studies however, will be necessary to more clearly define the role of NKT cells in asthma. These studies should include the examination of NKT cell number, phenotype and function before and after allergen challenge, examination of AHR after allergen challenge with and without blockade of NKT cell activation, analysis of the phenotype and the subsets of NKT cells present in the lungs in different forms of asthma, and analysis of NKT cells before and after viral exacerbations of asthma.

Summary and Conclusions

Bronchial asthma is complex problem, involving many different forms and pathogenic mechanisms. While the Th2 paradigm explains many features of asthma, there are many attributes and characteristics of asthma that are not related to Th2 cells, such as the presence in the lungs of neutrophils, IFN- γ , IL-17, and responses to viruses and oxidative stress/air pollution. Studies in mice and monkeys strongly suggest that many of these features might be explained by the presence of a newly described subtype of lymphocyte, called NKT cells. These studies show that distinct subsets of NKT cells are required for the development of AHR in several different models of asthma, involving adaptive and innate immunity (allergen-induced, ozone-induced and virus-induced AHR). These studies therefore greatly expand our understanding of several possible mechanisms that might drive the development of asthma, particularly in the case of asthma induced by viral infection and air pollution.

Validation of the concept that NKT cells contribute to asthma clearly requires additional human studies, since the current data for human asthma are incomplete, and definitive conclusions regarding the role of NKT cells in human asthma cannot yet be made. NKT cells are present in the lungs of many but not all patients with asthma, but such simple enumeration studies of NKT cells in the lungs of asthma patients are not sufficient. Nevertheless, the currently available studies of human asthma are very consistent with those in murine and non-human primate models and support an important role for NKT cells in human asthma. Moreover, future functional studies of NKT cells in humans with asthma will likely demonstrate the particular situations and mechanisms by which NKT cells modulate the development of AHR and asthma in humans.

Abbreviations

AHR	airway hyperreactivity
α -GalCer	α -galactocylceramide
BAL	bronchoalveolar lavage
DN	double negative
NKT	natural killer T
TCR	T cell receptor

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Table 1

Subsets of NKT cells involved in AHR responses

Subset	Model	Cytokines produced	Adaptive immunity required?	References
CD4 ⁺ , IL-17RB ⁺	Allergen-induced	IL-4, IL-13	yes	6, 7, 12, 38
DN, NK1.1 ⁻ , IL-17 ⁺	Ozone-induced	IL-17	no	10, 39, 40
DN	Sendai virus induced	IL13	no	11
Several subsets?	Glycolipid induced, α GalCer, Sphingomonas	IL-4, IL-13, IL-17	no	25, 40
Type II NKT cells*	Allergen induced AHR in β 2 microglobulin ^{-/-} mice	IL-4, IL-13	yes	20

DN=double negative (CD4⁻).

* Type II NKT cells express NKT cell characteristics, but not the invariant TCR.