

Natural Killer T Cells Regulate the Development of Asthma

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INTRODUCTION

Atopic diseases, which include asthma, allergic rhinitis, and atopic dermatitis, are complex genetic traits caused by environmental factors in genetically susceptible individuals. These disorders have increased dramatically in prevalence over the past two decades [1], such that roughly one in six individuals in the United States has some form of allergic disease. As a result, allergic diseases have reached epidemic proportions, and current health care expenditure for allergic disease, particularly asthma, is enormous [2]. The increase in prevalence of atopic diseases is thought to be due to changes in the environment that have occurred over the past 20 years in industrialized countries and not to be due to changes in the allele frequencies of the dozen or so polymorphic genes that regulate atopic diseases [3,4]. Nonetheless, environmental and genetic factors together result in the development of a Th2-biased immune response and the overproduction of Th2 cytokines such as IL-4, IL-5, IL-9, and IL-13 [5–7], which orchestrate the inflammation in allergy and asthma by enhancing isotype switch to IgE, enhancing the growth of eosinophils, basophils, and mast cells, and causing airway hyperreactivity (AHR), a cardinal feature of asthma.

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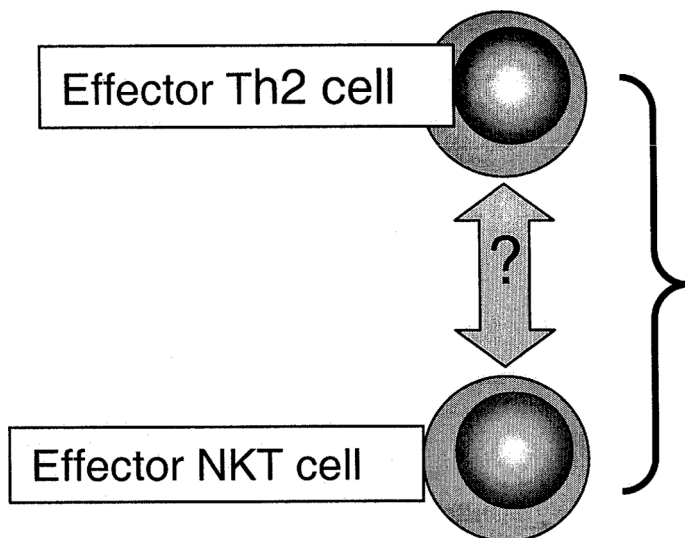


FIGURE 1 Th2 cells and NKT cells have overlapping phenotypes. Both produce IL-4 and IL-13 and express TCRs and the CD4 marker; both enter the pulmonary environment and can enhance the development of AHR. However, the activation requirements of these cell types are very distinct. Further study is required to understand the precise relationship between Th2 and NKT cells in the development of asthma.

Class II MHC restricted Th2 cells have been assumed to be the major source of Th2 cytokines in allergy. Th2 cells, which produce IL-4, IL-5, IL-9, and IL-13 and which are present in the lungs of patients with asthma, are thought to critically regulate allergic inflammation and to play an obligatory role in the pathogenesis of asthma [8]. However, NKT cells, which comprise a newly described, unique subset of lymphocytes that express features of both classical T cells and natural killer (NK) cells, are also capable of rapidly producing large amounts of Th2 cytokines. Moreover, a large fraction of natural killer T (NKT) cells, like conventional class II MHC restricted T cells, also express the CD4 cell surface marker. Recently, NKT cells have been shown to play a critical role in the development of asthma and airway AHR [9–12].

CHARACTERISTICS OF NKT CELLS

NKT cells comprise a heterogeneous group of cells that have characteristics of both natural killer cells and conventional T cells. NKT cells

can be divided into three major categories based on the repertoire of their T-cell receptors (TCRs) [13]. Type I (or classical) NKT cells expressing a highly restricted or conserved $\alpha\beta$ T-cell receptor (TCR) repertoire consisting of V α 14-J α 18 (in mice) or V α 24-J α 18 (in humans), and are called invariant (i) NKT cells [14]. Type II (or nonclassical or noninvariant) NKT cells have a diverse TCR repertoire but are restricted by CD1d. Type II NKT have been associated in human and mice with oxazolone-induced colitis in mice, ulcerative colitis in humans, or with suppression of tumor growth [15–18]. Type III (or CD1d-independent) NKT cells express a diverse TCR repertoire restricted by class I and II MHC molecules. Type III NKT cells express Ly49 or NK1.1 (CD161 in humans) and CD3 but cannot be identified with CD1d tetramers. In contrast, type I iNKT cells can be stained and isolated with CD1d tetramers loaded with α -galatosylceramide and appear to be critical for the development of asthma.

INVARIANT T-CELL RECEPTOR NKT CELLS

iNKT cells are CD4⁺ or CD4⁻ CD8⁻ (double negative), and in humans a small subset of iNKT cells express CD8. In contrast to the T-cell receptors (TCRs) of conventional class II MHC restricted T cells, which recognize peptide antigens in the context of class II MHC molecules, the invariant TCRs of iNKT cells recognize glycolipid antigens presented in the context of the nonpolymorphic MHC class I-like protein, CD1d [19–21]. CD1d is widely expressed, for example by intestinal and airway epithelial cells, T cells, hepatocytes, B cells, macrophages, and dendritic cells [19,22]. Recognition of CD1d-presented glycolipids, such as α -galactosylceramide (α -GalCer), by iNKT cells is highly conserved across phylogeny, suggesting that these cells play a pivotal role in immunity. The type 1 iNKT cells have been shown to be involved in asthma and AHR (see below).

The rapid production by iNKT cells of large quantities of cytokines such as IL-4 and IFN- γ within minutes of stimulation is a manifestation of innate-like immunity that can critically amplify and regulate adaptive immunity by enhancing the function of dendritic cells (DCs), NK cells, B cells, as well as conventional CD4⁺ and CD8⁺ T cells [13]. Moreover, the rapid production of cytokines by iNKT cells has been shown to regulate the development of autoimmune, antimicrobial, anti-tumor, and antitransplant immune responses [23], providing either disease-causing or disease-protective effects. For example, iNKT cells can either promote or prevent tumor growth, colitis, and experimental autoimmune encephalomyelitis (EAE) in mouse models. In the case of asthma, the production of IL-4 and IL-13 by iNKT cells is critical in the development of asthma.

NKT CELLS AND ASTHMA

In mice, several investigators demonstrated that iNKT cells were absolutely required for the development of AHR [9,11,12]. In these models, iNKT cell-deficient CD1d^{-/-} mice failed to develop allergen-induced AHR, as measured by whole-body plethysmograph or by direct measurement of airway resistance and dynamic compliance in intubated and mechanically ventilated mice, although they developed some level of eosinophilic inflammation. Wild-type mice treated with anti-CD1d blocking mAbs prior to allergen sensitization and challenge also failed to develop AHR [11], strongly suggesting that CD1d restricted iNKT cells were required for the development of asthma.

The requirement for iNKT cells in the development of AHR was confirmed by examination of allergen-induced AHR in another iNKT cell-deficient mouse strain, J α 18^{-/-} mice. These mice lack the α -chain of the invariant TCR and therefore specifically lack iNKT cells [24]. As with the CD1d^{-/-} mice, the J α 18^{-/-} mice failed to develop allergen-induced AHR as measured by whole-body plethysmography and by direct examination of airway resistance and dynamic compliance and developed reduced eosinophilic airway inflammation. Moreover, adoptive transfer of purified wild-type iNKT cells into the J α 18^{-/-} mice prior to allergen challenge fully reconstituted airway inflammation and AHR [9], indicating that iNKT cells were specifically required for the development of AHR. However, adoptive transfer of iNKT cells from IL-4^{-/-}IL-13^{-/-} into J α 18^{-/-} mice failed to restore AHR, indicating that the production by the iNKT cells of IL-4 and IL-13 but not IFN- γ was required for the development of AHR. Taken together, these studies, in several different laboratories in several different mouse strains using multiple different protocols, demonstrated that iNKT cells producing IL-4 and IL-13 were required for the development of allergen-induced AHR.

Although multiple studies have confirmed the requirement for iNKT cells in the development of AHR, there are some murine models of asthma in which iNKT cells have not been found to be necessary for the development of asthma. For example, one study showed that allergen-induced airway inflammation developed in CD1d^{-/-} mice, although there was a reduction in airway eosinophilia that did not reach statistical significance [25]. Importantly, AHR was not assessed in these studies but may be the critical feature that requires the presence of iNKT cells. In other studies using β_2 -microglobulin-deficient mice, iNKT cells appeared not to be required for the development of AHR. β_2 -microglobulin associates with CD1d α -chains to form the complete CD1d heterodimer molecule, and thus absence of β_2 -microglobulin

in these mice results in the lack all class I molecules including CD1d and absence of both CD8⁺ cells and iNKT cells. Sensitization and challenge of these β_2 -microglobulin-deficient mice resulted in airway eosinophilia and severe AHR [26,27]. However, CD1d-independent NKT are present in the β_2 -microglobulin^{-/-} mice [28,29], and some forms of CD1d are expressed in the absence of the β_2 -microglobulin chain [28,30], suggesting that some types of NKT cells may indeed be involved in the development of AHR in these mice. Nevertheless, it is possible that while iNKT cells are required for the development of allergen-induced AHR in most circumstances, there may be some situations in which some forms of airway inflammation or AHR may develop in the absence of iNKT cells.

iNKT CELLS, Th2 RESPONSES, AND IgE PRODUCTION

The requirement of iNKT cells for the development of AHR is curious, as antigen-induced Th2 responses and antigen-specific MHC II restricted CD4⁺ Th2 cells develop normally in CD1d^{-/-} and J α 18^{-/-} mice [9,31]. However, although iNKT cells are not required for Th2 responses, iNKT cells have been shown to play an important role in regulating Th2 immune responses [14]. For example, iNKT cells provide a very important source for Th2 cytokines and can modulate the development of Th2-polarized adaptive immune responses. Thus, investigators have found that coadministration of α -GalCer, which specifically activates iNKT cells, along with exogenous protein antigen results in enhanced sensitization of mice to these antigens [32]. These studies demonstrate that the activation of iNKT cells can function as an adjuvant early in the immune response and enhance the development of immunologic memory and adaptive immunity, which may contribute to allergen-induced AHR.

The induction of antigen-specific IgE synthesis during adaptive immune responses does occur in the absence of iNKT cells [31,33], as iNKT cell-deficient CD1d^{-/-} and J α 18^{-/-} mice developed robust antigen-specific IgE responses. However, treatment of β_2 -microglobulin^{-/-} mice with anti-IgD failed to induce IgE synthesis [34], and V α 14-J α 18 transgenic mice, which contain increased numbers of iNKT cells, exhibit significantly higher serum levels of Th2-associated immunoglobulins, IgG1 and IgE [35]. Similarly, activation of iNKT cells with α -GalCer or other glycolipid antigens results in a rapid rise in total serum IgE that remains elevated with chronic activation of iNKT cells with glycolipids [36]. These results suggest that iNKT cells may be involved in enhancing IgE production, either by providing a rapid source of IL-4 for isotype switch to IgE or by directly activating

B cells [37]. The precise mechanisms by which iNKT cells modulate IgE production, however, is currently poorly understood, and better understanding of this mechanism is likely to improve our understanding of hyper-IgE states and atopy.

ENDOGENOUS AND EXOGENOUS GLYCOLIPIDS AND THE INDUCTION OF AHR

In murine models of allergen-induced AHR, administration of protein antigens such as ovalbumin, bovine serum albumin, or ragweed antigen activates iNKT cells and induced the development of AHR. It is clear that iNKT cells do not recognize protein antigens, and therefore it is likely that endogenous glycolipids are released or expressed in the inflammatory environment generated by allergen-specific Th2 cells that activate iNKT cells, which then induce AHR. Thus, endogenous glycolipids, which serve as indicators of injured or altered self, might be expressed by both professional bone marrow-derived APCs as well as “nonprofessional” tissue cells such as hepatic or epithelial cells during inflammation. iNKT cells, expressing a restricted TCR repertoire, would then recognize these endogenous glycolipids, rapidly respond, and amplify this inflammatory signal.

The full range of endogenous glycolipid antigens is not yet known, but one important endogenous glycolipid, called isoglobotrihexosylceramide, or iGb3, has been identified [21,38]. iGb3 is crucial for the development of iNKT cells, because mice with a deficient β -hexaminidase b subunit cannot make iGb3 and show a 95% reduction in iNKT cell numbers. The identification of iGb3 as an endogenous glycolipid is an important advance, but iGb3 may be more important for the thymic maturation of iNKT cells, and other endogenous glycolipids may activate iNKT cells in the periphery. This may explain why intranasal administration of iGb3 does not induce AHR in mice in contrast with other glycolipid antigens, which activate iNKT cells (E.H. Meyer, unpublished observations). Nonetheless, iGb3 expression clearly occurs in an inflammatory setting. Thus, DCs pretreated with *Salmonella typhimurium* extract express iGb3 loaded into CD1d, which then activate iNKT cells [21,38]. The precise role of iGb3 in asthma, however, has not been determined, and other endogenous glycolipids, perhaps expressed primarily in the parenchyma in response to tissue injury or stress, may be as or more important in asthma.

Glycolipids that activate iNKT cells can also originate exogenously, for example from pathogens, as “non-self” antigens that bind to CD1d molecules and that activate the innate immune system [39,40]. Such pathogen-associated glycolipids that activate iNKT cells may be similar to pathogen-associated molecular patterns (PAMPs) recognized by

the innate immune system. However, in the case of glycolipids, the invariant TCR of iNKT cells functions as the pathogen pattern recognition receptor, which is not yet catalogued as an innate pathogen pattern recognition receptor. Pathogen-associated glycolipids include the bacterial membrane constituents from the Sphingomonadaceae and Rickettsiaceae families [20,38], many of which are gram⁻ LPS⁻ bacteria and include human bacterial pathogens such as *Sphingomonas paucimobilis* and *Erlchia chaffeensis*. Recently, it has been speculated that α -GalCer itself may in fact be derived from the Sphingomonadaceae, which infect marine sponges. The range of pathogens expressing glycolipids recognized by NKT cells is not known but may include *Borrelia burgdorferi*, *Cryptococcus neoformans*, *Plasmodium falciparum*, *Trypanosoma cruzi*, *Leishmania major*, and *Schistosoma mansoni* [20,40–45]. Whether pathogens encountered in the respiratory tract also express glycolipids recognized by NKT cells is not yet clear.

Plant pollens may also express exogenous glycolipids that directly activate iNKT cells. For example, cypress tree pollen contains lipids that have been shown to directly activate human iNKT cells *in vitro* [46]. The cypress tree pollen-derived lipids were recognized in the context of CD1a as well as CD1d and induced the proliferation of CD4⁺ $\alpha\beta$ TCR⁺, $\gamma\delta$ TCR⁺, and some V α 24⁺ iNKT cells. The cypress lipid responsive T cells could provide help for IgE production and were more evident in the peripheral blood of allergic subjects during the pollinating season. Finally, injection of the glycolipids induced cutaneous wheal and flare reactions indicating that anti-cypress lipid IgE antibodies were present in cypress allergic patients. These results suggest that plant pollens may be an important and previously unrecognized source of lipid antigens that directly activate iNKT cells, suggesting a mechanism by which pulmonary iNKT cells might become directly activated to cause allergy and asthma.

iNKT CELLS AS EFFECTOR CELLS IN THE DEVELOPMENT OF ASTHMA

As already discussed, iNKT cells are absolutely required for the development of AHR in many murine models of asthma and in addition serve as a source of large amounts of IL-4 and IL-13 to enhance antigen sensitization. Moreover, iNKT cells directly respond to exogenous glycolipid antigens from pathogens and pollens, suggesting that the activation of iNKT cells alone might be sufficient for the induction of AHR. Indeed, when pulmonary iNKT cells were directly activated by glycolipid antigens, they rapidly induced the development of AHR and airway inflammation in mice [36]. Thus intranasal administration of glycolipids that

activate iNKT cells (such as α -GalCer or a *Sphingomonas* glycolipid membrane constituent) resulted in the rapid induction of AHR, eosinophilic airway inflammation, and IgE production, which typify the Th2-driven inflammatory response normally associated with protein allergen-induced AHR. The induction of AHR with glycolipids did not require the presence of eosinophils (in mice treated with anti-IL-5 blocking mAb) or of B cells (in JHD B cell-deficient mice). Moreover, these glycolipids induced AHR in MHC class II^{-/-} mice that do not contain conventional CD4⁺ T cells but that have large numbers of iNKT cells, demonstrating that iNKT cell-driven AHR can occur in the complete absence of conventional CD4⁺ T cells and adaptive immunity.

Together these results indicate that iNKT cells are potent effector cells in the lungs and are entirely sufficient for inducing AHR. The iNKT cell effector function is distinct from the adjuvant effects of iNKT cells, in which iNKT cells provide large quantities of cytokines that affect adaptive immunity. Moreover, the direct induction of AHR by glycolipid antigens suggests that glycolipids from respiratory pathogens or from plants [46] might enter the lungs, directly activate iNKT cells, and drive the development of wheezing, airway inflammation, and asthma. Given evidence that iNKT cells are both necessary and sufficient for AHR, future studies examining the capacity of glycolipids from respiratory pathogens and plant pollens in activating iNKT cells and in inducing AHR could greatly alter our understanding of respiratory pathobiology.

Th2 CELLS IN ASTHMA

Although iNKT cells are both necessary and sufficient for the development of AHR, and although iNKT cells may be present in numbers comparable with conventional CD4⁺ T cells in the airways of patients with moderate to severe asthma (see below), conventional CD4⁺ Th2 cells also serve as important effector cells in asthma. Thus, in adoptive transfer models, Th2 CD4⁺ T cells act as effector cells in the induction of AHR [47–49], suggesting that both iNKT cells and Th2 cells share effector cell activity and the capacity to induce AHR. The precise relationship between iNKT cells and Th2 cells in asthma is not clear, but because Th2 cells recognize crucial environmental protein allergens that drive the development of allergic asthma, Th2 cells might enhance the production or expression of endogenous glycolipids, which then activate iNKT cells to induce AHR. Alternatively, iNKT cells may amplify the function of Th2 cells, perhaps by “licensing” conventional CD4⁺ Th2 effector cells to induce AHR [9]. This possibility is supported by the observation that pulmonary iNKT cells and conventional CD4⁺ T cells directly interact with each other [50]. Clearly, additional studies are

required to clarify the precise relationship between conventional CD4⁺ T cells and iNKT cells in the development of AHR and asthma.

iNKT CELLS IN HUMAN PATIENTS WITH ASTHMA

The studies of allergen-induced AHR in mice strongly suggested that iNKT cells might be important in human asthma. However, because murine models of asthma do not replicate all features of human asthma, the role of iNKT cells in humans with asthma cannot be precisely determined except by direct assessment of patients with asthma. Using CD1d-tetramers loaded with α -GalCer and RT-PCR analysis for the invariant TCR of iNKT cells, the frequency and distribution of iNKT cells in the lungs of patients with moderate to severe persistent asthma was determined [10]. Surprisingly, about 60% of the pulmonary CD4⁺CD3⁺ cells in the lungs of these patients with asthma were not class II MHC-restricted CD4⁺ T cells as has been thought, but rather iNKT cells. The NKT cells expressed the invariant V α 24⁺ TCR and produced IL-4 and IL-13, but not IFN- γ . In contrast, the CD4⁺ T cells found in the lungs of patients with sarcoidosis, an inflammatory disease in which large numbers of CD4⁺ Th1 cells are found in the lungs, were conventional CD4⁺CD3⁺ T cells and not iNKT cells. These studies strongly suggest that iNKT cells indeed play a prominent pathogenic role in human asthma.

The presence of large numbers of iNKT cells in the lungs of patients with asthma is surprising and suggests that iNKT cells may have been mistaken for conventional CD4⁺ Th2 cells in the past. Most of the iNKT cells in the lungs of patients with asthma expressed CD4 and produced IL-4, IL-13, but not IFN- γ , suggesting that a Th2-like subset of iNKT cells was recruited or expanded in the lungs of patients with asthma. Surprisingly, iNKT cells were not increased in the peripheral blood of patients with asthma, and peripheral blood iNKT had features similar to those from normal nonallergic, nonasthmatic individuals [10,51], indicating that the immunology of asthma must be studied not by the examination of peripheral blood alone but rather by the evaluation of cells from within the lung. Precisely how the CD4⁺ iNKT cell subset preferentially enters the lung, presumably from the peripheral blood, or what mechanisms preferentially expand the Th2-cytokine-producing iNKT cells in the lung are not yet clear.

In the limited number of patients with asthma studied so far, treatment with inhaled corticosteroids did not appear to affect the frequency of iNKT cells in the lungs [10]. This observation may be due to the fact that whereas conventional CD4 T cells are very sensitive to corticosteroid therapy, iNKT cells are relatively resistant [52,53].

The relative resistance of iNKT cells to corticosteroids and the importance of iNKT cells in the pathogenesis of asthma may explain the fact that 10–25% of patients with asthma respond poorly to corticosteroid therapy. These patients with corticosteroid-resistant asthma account for half or more of the health care costs related to asthma [54,55]. Further, because it is possible that the number of iNKT cells in the lungs correlates with disease severity, treatments for asthma that diminish the function or reduce the number of pulmonary iNKT cells may provide very effective therapy for asthma, particularly in those patients with the corticosteroid-resistant form.

THERAPIES TARGETING iNKT CELLS

Based on the idea that iNKT cells play such an important role in the development of asthma, several investigators have begun to demonstrate how inactivation of iNKT cells might form the basis for the treatment of asthma. One approach has been to block the activation of iNKT cells by blockade of CD1d with mAb. When given early during the development of allergen-induced AHR, anti-CD1d mAb prevents the development of AHR [11]. The effectiveness of this approach is limited, however, because it appears that iNKT effector cells, after initial activation, are less dependent on CD1d-TCR signaling and may function when anti-CD1d mAb is given later during the development of AHR (E.H. Meyer, unpublished observations). Another approach relies on α -GalCer administered intravenously or intraperitoneally 24 h prior to respiratory challenge of sensitized mice with OVA. This treatment reduces airway inflammation and inhibits allergen-induced AHR [56–58]. The administration of α -GalCer is thought to suppress AHR by enhancing the production of IFN- γ and/or the development of inhibitory/suppressor cells. In addition, because the administration of α -GalCer renders iNKT cells unresponsive to further stimulation for a period of days to weeks [59], α -GalCer treatment may involve iNKT cell anergy, thereby preventing iNKT cells from inducing AHR. Importantly, this therapeutic approach with α -GalCer may be problematic in that administration of α -GalCer may serve as an adjuvant to enhance allergen sensitization, thereby worsening allergy and AHR. Therefore, other therapeutic approaches focused on selectively eliminating iNKT cells in the lungs or selectively reducing iNKT cell function must be developed for optimal treatment of asthma.

THE Th2-LIKE iNKT CELL SUBSET

In general, activation of iNKT cells with α -GalCer results in the production of both Th1 and Th2 cytokines, and it has been unclear if subsets of iNKT cells producing restricted cytokine profiles could exist. The

unrestricted cytokine profile of iNKT cells is relatively fixed in mice, even in IL-4^{-/-} and IL-12^{-/-} mice, which lack Th2 and Th1 cells, respectively [60]. Moreover, resting V α 14i T cells from different organs of wild-type mice such as the liver and spleen contain mRNA for both IFN- γ and IL-4, and both types of cells produce both cytokines at the same ratios after *in vivo* activation with α -GalCer or other glycolipids. The persistence of a fixed cytokine profile in iNKT cells in contrast with polarized conventional T cells may be due to the fact that control of cytokine production in iNKT cells is distinct from that of conventional T cells. Notably, IL-4 production in iNKT cells does not require STAT6 signaling or the transcription factor GATA3 [61], and post-transcriptional regulation of IL-4 may be important, similar to situations in other cell types of the innate immune system like mast cells [62].

More recent studies of iNKT cells suggest that subsets of iNKT cells with restricted cytokine profiles do indeed exist. For example, human iNKT cells segregate into a CD4⁺ population, which produce both Th1 and Th2 cytokines (unrestricted profile) and a double-negative population (CD4⁻ CD8⁻) or CD8⁺ population, producing only Th1 cytokines and exhibiting greater cytotoxic activity [63,64]. In addition, a human CD4⁺ iNKT cell subset with the phenotype of Th2 cells producing only Th2 cytokines is present in the lungs of patients with asthma [10]. These Th2-like iNKT cells in the lungs may be related to their tissue location, especially as in mice that iNKT cell populations in different tissue locations appear to be discrete subpopulations based on their TCR β chain usage [65].

Studies of iNKT cells from specific anatomical locations in mice support the idea that functional subsets of iNKT cells indeed exist. Thus, liver-derived iNKT cells were shown to be distinct from those in the thymus or spleen in being better able to inhibit tumor growth [23]. Inhibition of tumor growth was associated with iNKT cells that did not produce IL-4, for example those that resided in the liver, but not in the thymus. In contrast, iNKT cells taken from the thymus of IL-4^{-/-} mice were more effective than thymic-derived WT iNKT cells in mediating antitumor responses. The anatomic location of the iNKT cells did not appear to alter the expression of CD4, which does not appear to segregate with cytokine profiles in mice, as it does in humans, although murine CD4⁺ iNKT cells may produce higher levels of IL-4 than CD4⁻ iNKT cells.

If a Th2-like subset is indeed important in asthma, the specific mechanisms by which Th2-like iNKT cells might develop are not yet understood. However, there are a number of observed associative factors that have been linked to the appearance of Th2-like iNKT cells that may be relevant. First, greater IL-4 with reduced IFN- γ production by iNKT cells is enhanced by chronic stimulation of iNKT cells. In many

situations, repeated dosing with α -GalCer causes iNKT cells to favor the production of IL-4 but not IFN- γ [66,67]. This may be related to the fact that immature murine iNKT cells produce more IL-4 than IFN- γ [68,69], and the replacement of older, anergic iNKT cells with fresh cells from the thymus could explain to some extent why chronic iNKT cell activation is associated with increased IL-4 production by these cells.

Second, analysis of glycolipid molecules structurally related to α -GalCer indicates that the manner in which iNKT cells are activated can affect how they produce cytokines and affect immune responses. Analogues with shorter fatty-acyl chains and sphingosine chains (e.g., OCH) induce greater amounts of IL-4 in iNKT cells [70]. The increased capacity of OCH to induce production of IL-4 is thought to be due to a reduced affinity of OCH for CD1d, resulting in reduced stability of the OCH-CD1d complex, and reduced interaction with the invariant TCR, akin to altered peptide ligands [71]. In contrast with OCH, another α -GalCer related molecule, α -C-GalCer (which has a single atomic substitution of C for O at the bond attaching the glycosyl head to the lipid tail), and other analogues more hydrophobic than α -GalCer may induce more IFN- γ than α -GalCer [72,73]. The capacity of α -C-GalCer to induce more IFN- γ , however, may be related to the capacity of α -C-GalCer to engage NK cells to produce IFN- γ . When NK cells are eliminated, the IFN- γ response to α -C-GalCer (and to α -GalCer) is greatly reduced. This is due to the fact that NK cells are stimulated to rapidly produce IFN- γ by CD8 α^+ dendritic cells (DCs), which have interacted with iNKT cells. In contrast with this interaction with DCs, studies have shown that when iNKT cells interact with B cells, iNKT cells produce IL-4 (weakly) rather than IFN- γ [74]. Thus, the type of and/or way in which APCs activate iNKT cells could elicit Th2-like iNKT cells.

Third, Th2-cytokine-producing iNKT cells are found in contexts where there is an increased homeostatic expansion of T cells, for example in transgenic mice expressing the invariant TCR of iNKT cells [35], or after transplantation conditioning regimes [75,76]. Finally, Th2-cytokine-producing iNKT cells appear especially linked to mucosal immune responses in mouse models of colitic inflammation and airway inflammation [9,77]. These general observations suggest that many factors that foster IL-4 $^+$ production in iNKT cells may be relevant in the development of asthma.

iNKT CELLS IN THE DEVELOPMENT OF OTHER ATOPIC DISORDERS

Atopic Dermatitis

The discovery that iNKT cells play an active role in asthma and AHR suggests that iNKT cells may be important in the context of other

allergic diseases. For example, atopic dermatitis is a chronic atopic inflammatory disease closely associated with allergic rhinitis and asthma that presents in infancy and afflicts between 10% and 20% of children and between 2% and 5% of adults in developed countries. A number of studies have examined iNKT cells in the peripheral blood of human individuals with atopic dermatitis, two of which found that patients with atopic dermatitis have fewer circulating $CD4^-CD8^-V\alpha24^+$ iNKT cells but roughly equivalent $CD4^+V\alpha24^+$ iNKT when compared with normal controls [78,79]. Another study found that patients with atopic dermatitis had more $CD4^+V\alpha24^+$ iNKT cells that produced IL-4 [80]. Although these studies may indicate an imbalance in $CD4^+$ iNKT cells in atopic dermatitis patients, trends in peripheral blood iNKT cells may not correlate with increases in iNKT cell numbers or CD4 expression at the actual anatomical site of allergic inflammation (e.g., in the skin). Further work in humans, particularly with samples from skin, will need to be performed to understand the role of iNKT cells in atopic dermatitis.

Contact Sensitivity

The role of iNKT cells has also been extensively examined in contact sensitivity (allergic contact dermatitis, contact hypersensitivity), a delayed-type hypersensitivity (DTH) reaction in the skin mediated by conventional $CD4^+$ T cells in response to environmental haptens that conjugate to self-protein antigens. Contact sensitivity cannot occur without the initial exposure to environmental haptens and sensitization of conventional $CD4^+$ T cells. This initial sensitization has been recently shown in mouse models of contact sensitivity to depend upon the presence of IL-4⁺ iNKT cells [81] and CD1d glycolipid presentation [82]. Both $CD1d^{-/-}$ and $J\alpha18^{-/-}$ mice fail to develop significant contact sensitivity, but contact sensitivity can be restored by adoptive transfer of wild-type but not IL-4-deficient iNKT cells into $J\alpha18^{-/-}$ mice [81]. Within 10 min of cutaneous exposure to hapten, iNKT cells in the liver produce IL-4. The IL-4 produced by iNKT acted on B1 B cells, which then produced IgM. This IgM, in turn, contributes to the homing and sensitization of conventional T cells in the skin. Without IL-4 from iNKT cells acting on B1 B cells, production of IgM and T-cell sensitization did not occur. The importance of iNKT cells in the priming phase of contact sensitivity is supported by experiments in which contact sensitivity was disrupted by the administration of lipid antagonists [polyethylene glycol (PEG)2000 dipalmitoyl-l- α -phosphatidylethanolamine (DPPE-PEG) and (PEG)2000 ceramide], which presumably disrupt glycolipid presentation by CD1d [82].

Colitis

Oxazolone-induced colitis is a mouse model of human ulcerative colitis in which production of IL-13 is required. iNKT cells mediate oxazolone-induced colitis, as deletion of iNKT cells, particularly those producing IL-13, prevents oxazolone-induced colitis from developing [77]. Moreover, NKT cells isolated from the lamina propria of the intestines of patients with ulcerative colitis produce IL-13. The NKT cells from patients with ulcerative colitis are type II NKT cells, as they do not express the invariant TCR of NKT cells and are cytotoxic for intestinal epithelial cells [17]. These studies demonstrate that ulcerative colitis is associated with an atypical Th2 response mediated by type II NKT cells producing IL-13. In contrast, other models of colitis are associated with a Th1 response, in which NKT cells may provide protection. For example, in a dextran sodium sulfate model of colitis, which is associated with Th1-type colitis, treatment with α -GalCer induces IL-4 and IL-10 production, which in this case protects against colitis [83]. More work is needed to further characterize iNKT cells or type II CD1d-restricted iNKT cells that infiltrate the intestinal mucosa in patients with colitic diseases.

CONCLUSION

During the past 5 years, it has become clear that invariant TCR⁺ CD1d-restricted iNKT cells (iNKT or type I NKT cells) play a very important role in Th2 inflammation and asthma. iNKT cells function to skew adaptive immunity toward Th2 or act directly as effector cells in mediating mucosal allergic responses in bronchial asthma and in contact hypersensitivity, whereas type II NKT cells appear to play an important effector cell role in ulcerative colitis. However, much more work remains to be done in examining and further characterizing the role of iNKT cells in allergic diseases, particularly the role and characteristics of Th2-like subsets of iNKT cells, their relationship to conventional CD4⁺ T cells, and defining of the glycolipids to which they respond. We believe that a greater understanding of the mechanisms governing the function of iNKT cells will contribute to a greater comprehension of asthma and to the development of new therapeutic approaches for asthma and possibly for other allergic diseases.

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