

Role of NKT cells in allergic asthma

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T helper 2 (Th2) cells play crucial roles in the development of allergic asthma, while various distinct cell populations also contribute to the pathogenesis of the disease. Invariant natural killer T (iNKT) cells produce large amounts of cytokines such as IL-4 and IFN γ upon stimulation with a ligand, α -galactosylceramide, and regulate various immune responses. Recently, a critical role of iNKT cells in the mouse model of asthma and also in asthma patients has been reported, while some contradictory results have also been described. Here, we summarize the experimental results in mouse and human systems, and discuss the current understanding of the role of NKT cells in the pathogenesis of asthma, including a possible mechanism by which iNKT cells are activated in asthma patients.

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Current Opinion in Immunology 2010, **22**:807–813

This review comes from a themed issue on
 Allergy and hypersensitivity
 Edited by Marsha Wills-Karp and Martien Kapsenberg

Available online 4th November 2010

0952-7915/\$ – see front matter
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DOI [10.1016/j.coi.2010.10.008](https://doi.org/10.1016/j.coi.2010.10.008)

Introduction

It is well established that CD4⁺ T helper 2 (Th2) cells play an important role in allergic disorders by producing Th2 cytokines [1]. IL-4 induces antigen-specific IgE production from B cells, and IgE and antigen activate mast cells to release various chemical mediators. IL-5 induces the development, activation and survival of eosinophils, while IL-13 induces airway hyperreactivity (AHR) and mucus hyper production [2]. Animal studies investigating these processes are very helpful to explain the mechanisms underlying the development of allergic asthma. However, in asthma patients, several types of therapies targeted to these Th2 cytokines were not as effective as many investigators expected [3]. Indeed many patients show a non-allergic form of asthma in which no allergen-specific IgE are detected. Furthermore, it is well known that Th2 cell-independent factors such as viruses, air pollution, and exercise induce and/or

exacerbate asthma symptoms. In addition, it has been reported that non-Th2 factors such as IFN γ , IL-17, and neutrophils contribute to severe asthma and steroid-resistant asthma [4,5]. These results indicate that Th2 responses are not obligatory in the development of asthma.

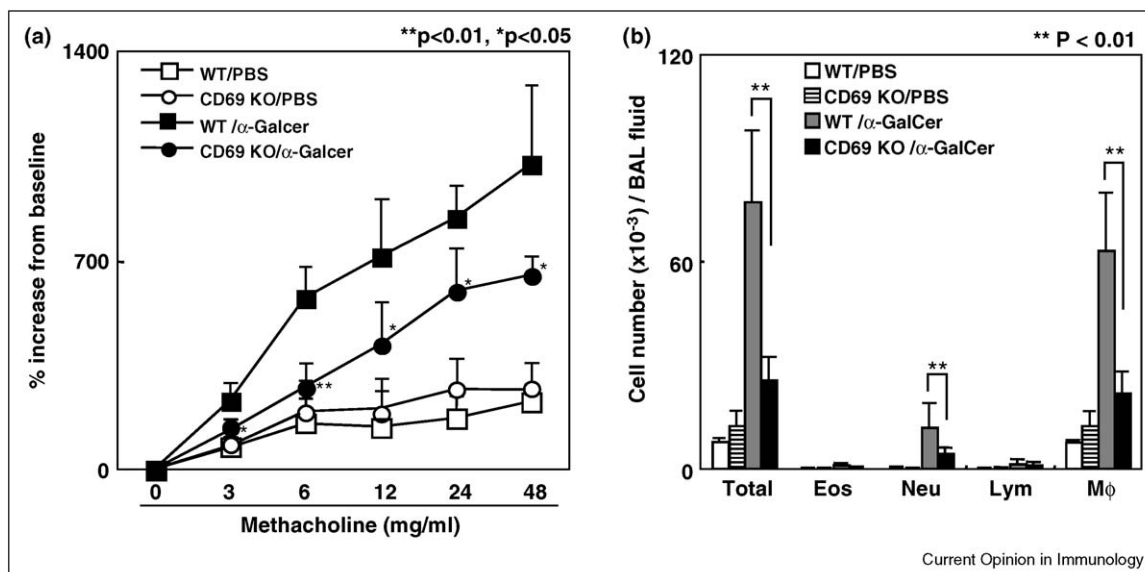
Natural killer T (NKT) cells belong to a novel lymphoid lineage distinct from T cells, B cells or NK cells. NKT cells are characterized by the expression of a restricted repertoire of T cell receptors (TCRs) consisting of V α 14 and J α 18 (in mice) or V α 24 and J α 18 (in humans) [6]. These receptors are associated with a highly skewed set of V β s, mainly V β 8.2 (in mice) and V β 11 (in humans). Since these TCRs are highly restricted, NKT cells having these receptors are called invariant NKT cells (iNKT cells). The most potent and well-analyzed ligand for the iNKT antigen receptor is a glycolipid, α -galactosylceramide (α -GalCer), which is exclusively presented by CD1d, a monomorphic class Ib molecule. Activated iNKT cells play critical roles in the regulation of various immune responses, such as allergic inflammation, anti-tumor immunity and autoimmune responses.

Upon activation, iNKT cells produce a large amount of both type 1 and type 2 cytokines, that is, IFN γ and IL-4/IL-5/IL-13. Therefore, iNKT cells may inhibit or exacerbate allergic responses. The activation of iNKT cells with α -GalCer at the sensitization phase attenuated allergic airway inflammation and Th2 responses via production of IFN γ [7,8,9^{*}]. In these reports, iNKT cells showed a suppressive function rather than promoting disease. On the other hand, many investigators have examined whether experimental allergic asthma is induced in iNKT cell-deficient mice or whether the activation of iNKT cells in the lung results in the development of asthma symptoms in mice. iNKT cells from patients with asthma were also assessed to reinforce the relationship between iNKT cells and allergic disease. In human studies, both positive and negative results regarding the contribution of iNKT cells in asthma were reported [10^{**},11^{**}]. We herein focus on the role of iNKT cells in the pathogenesis of asthma in animal models and discuss the contribution of iNKT cells to human asthma.

Do NKT cells induce experimental allergic asthma?

Activated iNKT cells may induce allergic asthma symptoms independently from Th2 cells. To address this question, Akbari *et al.* assessed OVA-induced airway hyperreactivity (AHR) and airway inflammation in iNKT cell deficient J α 281 KO and CD1d KO mice. This study

Figure 1



iNKT cell-dependent AHR and airway inflammation were attenuated in CD69-deficient mice. To examine the role of CD69⁺ iNKT cells in NKT cell-dependent asthma, 1.5 μ g of α -GalCer was administered intranasally into wild type mice (WT) and CD69-deficient (KO) mice. **(a)** AHR in WT and CD69 KO mice 24 hours after α -GalCer treatment. Airway resistances were assessed in an invasive system as described previously [46]. **(b)** Decreased infiltrated leukocytes in BALF of CD69 KO mice after intranasal administration of α -GalCer. 24 hours after α -GalCer treatment, BALF of WT and CD69 KO mice were collected. Mean values (5 mice per group) are shown with SDs. * $P < 0.05$ and ** $P < 0.01$, student t test.

showed a significant defect in the development of AHR and inflammation in the absence of iNKT cells [12]. The authors concluded that iNKT cells are required for the development of AHR and airway inflammation. The administration of anti-CD1d antibodies or a CD1d-dependent antagonist can also suppress OVA-induced AHR and airway inflammation [13,14^{*}]. The respiratory administration of α -GalCer rapidly induced AHR and inflammation in naïve mice and this effect was seen even in naïve MHC class II-deficient mice, which lack conventional CD4 T cells [15]. In addition, a particular subset of iNKT cells that lack the NK1.1 marker produced IL-17 and induced neutrophilic infiltration following the intranasal administration of α -GalCer [16]. We recently reported that CD69⁺ Th2 cells play a crucial role in OVA-induced airway inflammation and AHR [17]. As shown in Figure 1, α -GalCer-induced AHR and leukocyte infiltration, including neutrophilic infiltration was attenuated significantly in CD69-deficient mice, indicating that activated iNKT cell-mediated asthmatic responses were dependent on CD69. Thus, CD69⁺ iNKT cells may play a critical role in the development of AHR and airway inflammation. Matangkasombut *et al.* demonstrated that the direct activation of pulmonary iNKT cells with α -GalCer in non-human primates resulted in the development of AHR, indicating that pulmonary iNKT cells are critical effector cells in this model [18].

However, there are several reports indicating that iNKT cells are dispensable for allergic airway inflammation.

Allergic inflammation occurred normally under certain conditions in CD1d-deficient mice and β 2-microglobulin (β 2m) KO mice that lack iNKT cells [19]. More recently, however, Koh *et al.* found that AHR can develop in β 2m KO mice [20]. In this report, they concluded that non-classical NKT cells, which are restricted to a β 2m-independent form of CD1d contribute to the development of AHR. Although the reason is not clear, these discrepancies in the results obtained from β 2m KO mice may be due to the difference in the experimental systems used.

iNKT cells in asthma patients

In order to identify the possible role of iNKT cells in human asthma, several investigators assessed the number of iNKT cells in asthma patients (Table 1). An initial report was published in 2006 by Akbari *et al.* reporting that more than 60% of CD4 T cells in the bronchoalveolar lavage fluid (BALF) from asthmatic patients were iNKT cells, while NKT cells were not observed in patients with sarcoidosis or in healthy controls [21]. Two other groups published supportive reports in the same year [22–24].

However, four other groups performed similar studies and concluded that iNKT cells did not increase in the patients with asthma [25–29]. Vijayanand *et al.* reported that iNKT cells were found in low numbers in the airways of patients with asthma, COPD or healthy controls with no significant differences between the three groups [26]. Mutalithas *et al.* also reported similar results in the BALF [27]. Moreover, the influx of iNKT cells into the airways

Table 1

iNKT cells in patients with asthma

Year	iNKT cells are			
	pathogenic		not pathogenic	
2006	Akbari	About 60% of CD4 ⁺ CD3 ⁺ cells in BALF from patients with severe asthma were CD1d-restricted NKT cells [21]	Thomas	Only 0.4–2.1% of lymphocytes in BALF of asthmatic patients were 6B11+ NKT cells [25]
	Pham-ti	The frequency of CD1d-restricted NKT cells in BALF from severe asthmatic children was higher than in BALF from controls [22,23]		
	Hamzaoui	The number of CD3 ⁺ CD56 ⁺ NKT cells in the sputum of severe asthmatic patients is increased as compared to that of controls [24]		
2007			Vijayanand	Fewer than 2% of CD4T cells were CD1d-restricted, 6B11 ⁺ or V α 24 ⁺ NKT cells in airway biopsy, BALF, and sputum of mild or moderately severe asthmatic patients and controls [26]
			Mutalithas	The presence of 6B11 ⁺ NKT cells in BALF of mild asthmatic patients was not significantly higher than that of controls [27]
			Thomas	Approximately 1% of lymphocytes were 6B11 ⁺ V α 24 ⁺ NKT cells in BALF and this did not increase with an antigen challenge [28]
			Bratke	Less than 1% of T cells were 6B11 ⁺ NKT cells in BALF of mild asthma patients [29]
2009	Matangkasombut	CD1d-restricted NKT cells in BALF of severe or mild asthmatic patients increased (readdressed) [30**]		
	Reynold	CD1d-restricted NKT cells increased in lung specimens from mild asthmatic patients as compared to those from controls [31]		
2010	Koh	V α 24 ⁺ or 6B11 ⁺ NKT cells were significantly increased in sputum from patients with asthma as compared to those from controls [32]		

BALF, bronchoalveolar lavage fluid.

was not observed after segmental allergen challenge [28,29].

By contrast, all studies we have identified from 2008 emphasized the existence of, and an increase in iNKT cells in patients with asthma [30**,31,32]. Matangkasombut *et al.* readdressed the issue regarding the number of iNKT cells in BALF from patients with severe asthma [30**]. They confirmed that patients with severe asthma had a significant increase in the number of iNKT cells as compared to healthy controls. In this report, however, the numbers of iNKT cells were 2–7% of total CD3⁺ cells in BALF of asthmatic patients. Only one patient with severe asthma had 64.5% NKT cells. Reynolds *et al.* supported this idea using lung biopsies with allergen challenge [31]. At present, it is unclear why studies on iNKT cells in asthma patients have provided divergent results. However, as the field matures it is becoming more evident that iNKT cells likely do play a role in the development and possibly exacerbation of allergic asthma. In addition, the

studies of iNKT cells in other asthma etiologies, such as chronic, occupational, steroid-resistant, exercise-induced, and aspirin-induced asthma, where Th2 cells may not play a major role, could provide new insights into these types of diseases.

What activates iNKT cells *in vivo*?

Even if activated iNKT cells can cause and exacerbate allergic asthma, α -GalCer is a component of a marine sponge and it is very rare to be exposed to α -GalCer during daily life. Respiratory organs are continually exposed to environmental stimuli including allergens, pathogens, and air pollution. Several studies indicate that substances naturally occurring in our environment may activate iNKT cells and develop or exacerbate allergic airway inflammation.

The invariant TCR of iNKT cells recognizes glycolipids from bacteria such as *Sphingomonas*, *Borrelia*, and *Leishmania* species [33]. Glycolipids purified from *Sphingoma-*

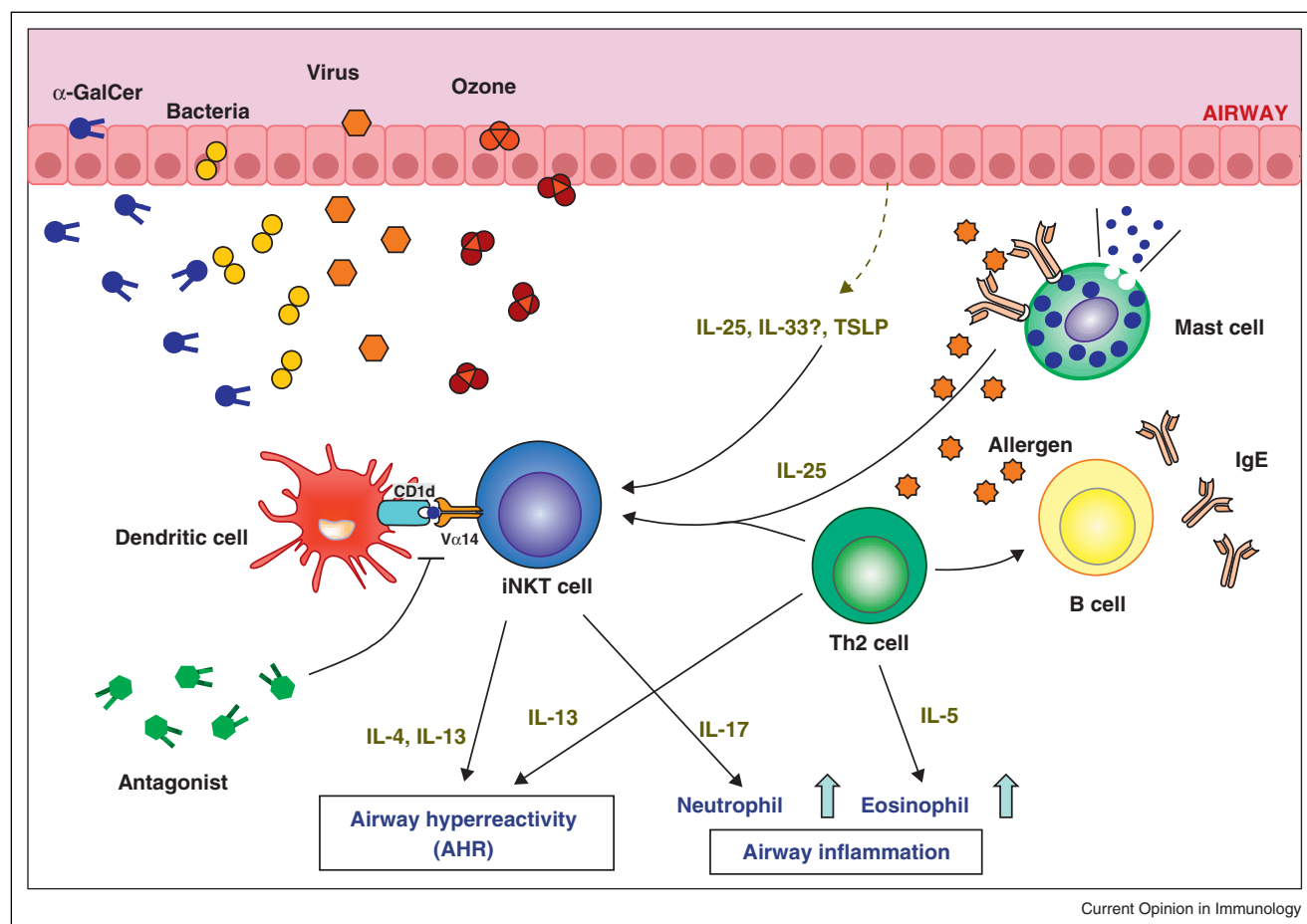
nas cell wall induced rapid AHR after respiratory administration in WT mice but not iNKT-deficient mice [15]. Once iNKT cells are activated by virus antigen, they stimulated macrophages to produce IL-13, resulting in the development of AHR and mucus production independently from the adaptive immune response [34]. It is well known that respiratory infection exacerbates the symptoms of allergic diseases under certain conditions. Therefore, iNKT cells activated by glycolipid from pathogens may contribute to the development and exacerbation of asthma symptoms in humans.

Ozone, as an air pollutant, has been reported to be associated with asthma [35,36]. Even in healthy individuals, exposure to ozone resulted in the development of AHR, associated with airway epithelial cell damage and increased numbers of neutrophils. Asthmatic patients appeared to be more susceptible to the adverse effects of this pollutant. Pichavant *et al.* showed that iNKT cells

were required for ozone-induced asthma through production of IL-17 in the mouse lung [37]. Although it is not clear how ozone activates iNKT cells, NKT cells activated by ozone can induce a form of asthma that is characterized by neutrophilic infiltration and AHR.

Recently, several cytokines involved in the initiation and amplification of Th2 responses were reported [38]. IL-25 also known as IL-17E, a member of the structurally related IL-17 cytokine family, is produced by activated Th2 cells, epithelial cells, basophils, and mast cells and is capable of enhancing AHR. Administration of recombinant IL-25 induced Th2 type responses, including increased serum IgE levels, eosinophilia, pathological changes in the lung, and AHR. These symptoms induced by IL-25 were not observed in iNKT cell deficient mice [39,40]. Transfer experiments of iNKT cells showed that iNKT cells expressing IL-17 receptor B (IL-17RB) are essential for IL-25-induced AHR. Thymic

Figure 2



Activated iNKT cells and Th2 cells in the development of AHR and airway inflammation. Th2 cytokines produced by antigen-specific Th2 cells can cause allergic asthma phenotypes through IgE induction, mast cell activation, eosinophilic infiltration into the lung, and AHR. Lung iNKT cells are activated by environmental substances or pro-Th2 cytokines. The activated iNKT cells induce AHR and infiltration of neutrophils predominantly in the airway by producing IL-13 and IL-17, respectively.

stromal lymphoprotein (TSLP) is produced by epithelial cells, mast cells, and basophils, and is considered to play an important role in experimental models of asthma. The targets of TSLP are T cells, mast cells, basophils and dendritic cells (DCs). In addition, Nagata *et al.* showed that TSLP also acts on iNKT cells to enhance AHR by up-regulating their production of IL-13 [41]. IL-33 is an IL-1 family member whose expression is also increased in epithelial cells in asthmatic patients. IL-33 enhanced the production of Th1 and Th2 cytokines in activated iNKT cells [42,43]. Based on these results, natural ligands in the environment may activate iNKT cells to induce allergic asthma, and pro-Th2 cytokines such as IL-25, TSLP and IL-33 may exacerbate the allergic symptoms.

How do iNKT cells control Th2 immune responses?

One enigma is the dynamics of iNKT cell accumulation in the lung during airway inflammation. Since the number of iNKT cells in the lung, lymphoid organs and PBMCs of humans and mice is very low (<0.1%) as compared to that of T cells or NK cells [44], it is curious how iNKT cells contribute to asthmatic symptoms. It is not known whether iNKT cells are activated and proliferated in the asthmatic lung or if activated iNKT cells migrate from lymphoid organs to the lung. We recently visualized the migration of antigen-specific Th2 cells into the lung in living mice after antigen exposure in the airway [45]. Using this *in vivo* live imaging system, visualization of the migration and localization of iNKT cells in the lung may provide important insights into these questions. In addition, possible cell–cell interactions between iNKT cells and various types of APCs, epithelial cells or Th2 cells in the asthmatic lung would be another interesting issue that may be clarified using *in vivo* imaging systems.

Conclusion

Figure 2 illustrates the role of activated iNKT cells and Th2 cells in AHR and airway inflammation. Both activated iNKT cells and Th2 cells produce various cytokines in the lung and induce AHR and airway inflammation. In the murine experimental model of asthma, activated iNKT cells appear to contribute as effector cells and also as an amplifier of allergen-specific Th2 cell responses. Although these mechanisms clarified in mouse models have not been directly investigated in asthmatic patients, NKT cells may yet prove to be a new therapeutic target for allergic diseases.

Acknowledgements

The authors are grateful to Drs. Damon Tumes and Yuki Kinjo for valuable comments during the preparation of this manuscript. This work was supported by Global COE Program (Global Center for Education and Research in Immune System Regulation and Treatment), and by grants from the Ministry of Education, Culture, Sports, Science and Technology (Japan) (Grants-in-Aid: for Scientific Research on Priority Areas #17016010; Scientific Research (B) #21390147, Young Scientists (B) #22790452), and the Ministry of Health, Labor and Welfare (Japan).

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