

# Invariant Natural Killer T (iNKT) Cells in Asthma: A Novel Insight into the Pathogenesis of Asthma and the Therapeutic Implication of Glycolipid Ligands for Allergic Diseases

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## ABSTRACT

Allergic bronchial asthma is a complex inflammatory diseases originated from dysregulated immune responses in the respiratory mucosa. The inflammatory state in asthmatic lung is characterized by massive infiltration with eosinophils, lymphocytes, and mast cells in the airway mucosa leading to airway hyperseisitivity, goblet cell hyperplasia and mucus overproduction. The inflammatory process is thought to be the result of intensive T helper (Th) 2-biased immune response. Over the past several years, there has been enormous progress in understanding the mechanisms for development of Th2-biased responses after inhaled exposure to allergens and the characteristics of CD4+ T cells prominently involved in this process. Recently, a new population of T cells, invariant natural killer T (iNKT) cells has been shown to play an important role in the pathogenesis of mouse model of allergic airway inflammation. iNKT cells are one of the most potent immune modulators through a massive production of a various cytokines including IL-4 and IFN- $\gamma$  upon activation, and are involved in a variety of immunoregulations including infection, autoimmunity, and tumor surveillance. The potent pathogenic role of iNKT cells in the development of bronchial asthma is due to their ability to produce predominant Th2 cytokines in a given condition. The involvement of iNKT cells in the pathogenesis of asthma might have been underestimated in the past studies demonstrating the involvement of CD4+ T cells in asthma because of the difficulty in the detection of iNKT cells. Meanwhile, growing evidences have demonstrated that iNKT cells could be a promising target for immune-based therapies for autoimmune diseases, tumor, and infection due to the invariance of their TCR usage, the restriction to the evolutionally-conserved non-polymorphic antigen-presenting molecule CD1d, and their outstanding ability to produce both Th1- and Th2-cytokines. In this review, we will overview current understanding of the pathophysiological roles of iNKT cells in asthma. We would also discuss on possible therapeutic approaches to bronchial asthma employing glycolipid ligands for iNKT cells.

## KEY WORDS

allergic asthma, glycolipid ligand, invariant NKT cells, Th2 cytokine, T helper (Th) 2 cell,  $\alpha$ -galactosylceramide

## INTRODUCTION

Accumulating evidence has verified the critical role of the cytokine milieu in a wide variety of immune responses *in vivo*. Especially, Th1 cells producing IFN- $\gamma$ , IL-2, and TNF- $\alpha$  are important for cell-mediated immunity and eliminate intracellular pathogen. Meanwhile, Th2 cells producing IL-4, IL-5, and

IL-13 control humoral immunity to helminthic parasites and are believed to be responsible for initiation and perpetuation of allergic asthma.<sup>1</sup> In addition, IFN- $\gamma$  and IL-4 themselves are involved in the differentiation of Th1 and Th2 cells, respectively<sup>2</sup> and these two subsets of helper T cells cross-regulate each other. Therefore, fine-tuning of Th1/Th2 balance is essential for avoiding host-threatening immune dysfunction.

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tion. Excessive Th1- and Th2-response to specific antigens has been shown to cause the process leading to the pathogenesis of autoimmune or allergic diseases, respectively. Allergic bronchial asthma is a complex inflammatory disease caused by superfluous immune response to inhaled exogenous allergen in the respiratory mucosa.<sup>3</sup> The inflammatory state in asthmatic lung is characterized by massive infiltration with eosinophils, lymphocytes, and mast cells in the airway wall concomitant with a various Th2 cytokines such as IL-4, IL-5, and IL-13, which orchestrate the recruitment and activation of T cells, B cells, eosinophils, and mast cells. There has been tremendous progress in understanding the mechanisms for development of Th2-biased responses after inhaled exposure to allergens and the characteristics of Th2 cells prominently involved in this process. For example, IL-13 has been shown to be a potent initiator of an inflammatory response, goblet cell hyperplasia, airway hyperresponsiveness (AHR), and airway fibrosis.<sup>4</sup> More recently, two molecules, thymic stromal lymphopoietin (TSLP)<sup>5,6</sup> and Interleukin-25 (IL-25),<sup>7,8</sup> are shown to be relevant to the development of Th2-driven inflammation and the pathogenesis of asthma. Furthermore, the studies on protective immunity to asthma have been focusing on the role of TGF- $\beta$  expressed on regulatory T cells and following activation of Notch/HES-1 signaling on tolerance induction in the lung.<sup>9</sup> Recently, a new population of T cells, invariant natural killer T (iNKT) cells has been shown to be involved in the pathogenesis of asthma possibly by enhancing Th2 response in animal models of allergic airway inflammation and in asthma patients.<sup>10</sup> Current understanding of the pathophysiological roles of iNKT cells in asthma and possible therapeutic approaches employing glycolipid ligand for iNKT cells will be overviewed and discussed in the following sections. The elucidation of the precise immunological relevance of iNKT cells in allergic asthma will be helpful to yield new immunotherapeutic strategies for allergic diseases.

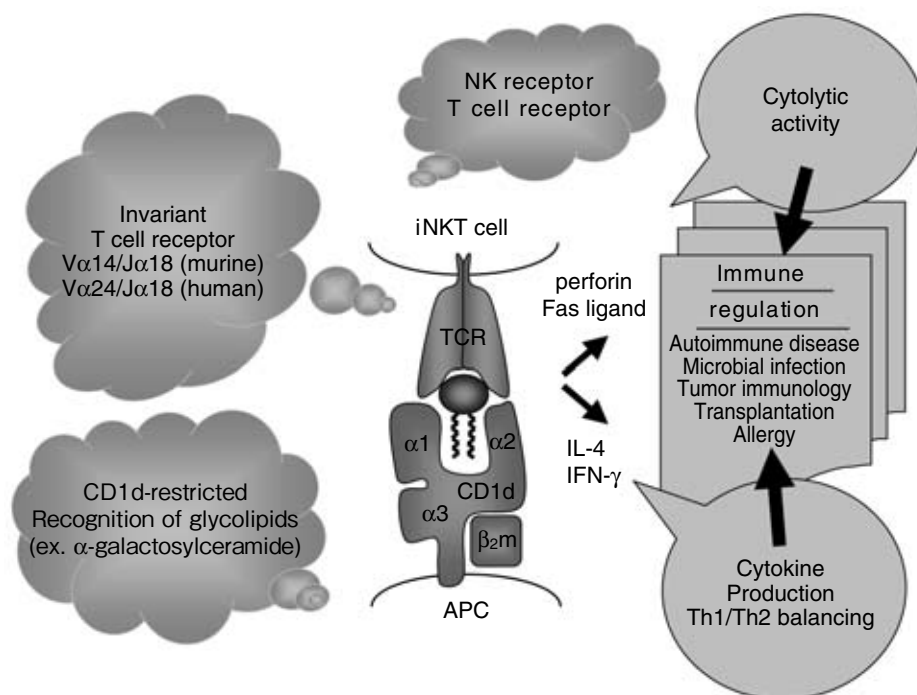
### **CONVENTIONAL TH2 CELLS IN ASTHMA DEVELOPMENT**

In the conventional instructive model of helper T cell differentiation, IL-12 and IFN- $\gamma$  induces the differentiation of Th1 cells and IL-4 has an essential role for transition from Th0 cells to Th2 cells, which provide a clear prospect for molecular mechanism of immune deviation. Consequently, helper T cell differentiation turned out to be unexpectedly flexible and to be established after stimulation by both specific antigen and cytokine microenvironment. Although recent studies demonstrated that Th1 cells do not always cross-regulate Th2 cells,<sup>11</sup> considerable evidence suggests that CD4<sup>+</sup> T cells and Th2 cytokines are major players in the pathogenesis of airway inflammation in asthma.<sup>12</sup> Elevated numbers of T cells have been

identified in the bronchoalveolar lavage (BAL) fluid and bronchial biopsy specimens from asthmatic patients and these T cells are predominantly CD4<sup>+</sup> T cell subset. Direct evidence of a pathogenic role for CD4<sup>+</sup> T cells in the development of asthma was first demonstrated in a murine model of allergen-induced allergic airway inflammation, where *in vivo* depletion of CD4<sup>+</sup> T cells by anti-CD4 monoclonal antibody before antigen challenge prevented both airway AHR and eosinophilic infiltration.<sup>13</sup> CD4<sup>+</sup> T cells increased in the airway of asthma patients express activation markers including CD25 and MHC class II and produce IL-4, IL-5, and IL-13. These cytokines maintain and amplify the Th2 polarization of T cell response, stimulate class switching of B cells to IgE production, and promote eosinophilic expansion and activation, leading to asthma. Intriguingly, a number of studies pointed out that most of phenotypic characteristics of conventional CD4<sup>+</sup> T cells described above are applicable to those of iNKT cells. Therefore, the involvement of iNKT cells in asthma may have been underestimated in the past studies. The authors will review the general property of iNKT cells in the next section.

### **VERSATILE INKT CELLS: MULTI-POTENTIAL IMMUNOMODULATOR**

iNKT cells constitute a unique and evolutionally-conserved subpopulation of T lymphocytes that express both T cell receptor (TCR) and NK receptors such as NK1.1 or Ly49 in mice and CD161 in human.<sup>14-16</sup> iNKT cells recognize glycolipid antigens by an invariant TCR $\alpha$  chain composed of V $\alpha$ 14-J $\alpha$ 18 segments in mice and V $\alpha$ 24-J $\alpha$ 18 segments in humans, and is associated with TCR $\beta$  chains using a restricted set of V $\beta$  genes (Fig. 1). Experimentally, these cells could be detected by staining with CD1d tetramer preloaded with  $\alpha$ -galactosylceramide ( $\alpha$ GC), which is currently prerequisite for describing the term 'iNKT cells'.<sup>17</sup> Most of iNKT cells are either CD4<sup>+</sup> or double negative (DN) and are characterized by a pre-activated phenotype, being CD69<sup>+</sup>, CD62L<sup>low</sup>, and CD44<sup>high</sup> and secrete a large amount of cytokines including IL-4 and IFN- $\gamma$  in a few hours after stimulation with either by anti-CD3 antibody or  $\alpha$ GC *in vivo*, the feature of which is a good contrast with conventional T cells quiescent in the absence of encounter with specific antigens *in vivo*. In fact, activated iNKT cells are one of the most potent immune modulators through a massive production of a various cytokines including IL-4 and IFN- $\gamma$  and were the major source of cytokines among TCR-bearing cells after stimulation with anti-CD3 antibody *in vivo*. iNKT cells have been shown to recognize both exogenous and endogenous glycolipid molecules with a variety of structural trait. The most potent glycolipid antigens for iNKT cells to date is  $\alpha$ GC,<sup>18</sup> a marine sponge-derived glycosphingolipid with immunomodulatory activities that is being exploited for immune-based therapeutic



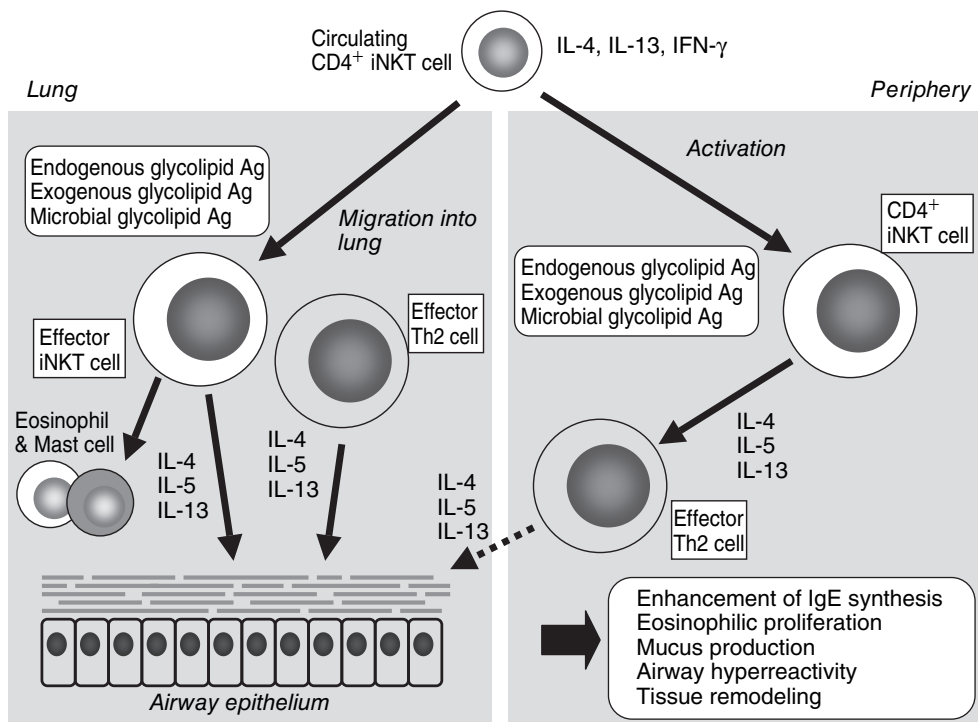
**Fig. 1** Phenotypic overview of iNKT cell and its suggested functional properties. NKT cells express invariant TCR  $\alpha$  chain ( $V\alpha 14$  in mouse and  $V\alpha 24$  in human) and recognize glycolipid ligand presented by CD1d on antigen presenting cells. iNKT cells are potent to produce a wide variety of cytokines, which enable iNKT cells to be involved in many immunoregulatory phases including allergy.

intervention of diseases. In addition to their conserved TCR usage, wide-spread distribution in all individuals, and their potent ability to produce a variety of cytokines, iNKT cells recognize glycolipid antigens in the context of MHC class I-like molecule, CD1d, which is non-polymorphic and well-conserved both in mouse and human.<sup>19</sup> Therefore, iNKT cells could be a promising target for immune-based therapies for autoimmune diseases, tumor, and infection (Fig. 1), if a proper manipulation of pleiotropic functions of iNKT cells is available, for example, by modifying the structure of prototypic glycolipid antigen,  $\alpha$ GC. The authors have previously demonstrated that OCH, a sphingosine-truncated analogue of  $\alpha$ GC, preferentially induced Th2 cytokines from iNKT cells and administration of OCH suppressed Th1-dominant autoimmune disease models such as experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis (CIA), type I diabetes (T1D) in non-obese diabetes (NOD) mice, and dextran sulfate sodium (DSS)-induced experimental colitis by inducing a Th2-bias of autoantigen reactive T cells.<sup>20-23</sup> Selective regulation of cytokine production from iNKT cells by using modified glycolipid ligands may expand the possible application of glycolipid therapy beyond autoimmune diseases.<sup>24</sup>

Besides the notion that iNKT cells are one of the

most attractive targets for manipulation of immune response by appropriated glycolipid antigens *in vivo*, there has also been tremendous progress in understanding pathophysiological roles of iNKT cells by analyzing immune responses in  $J\alpha 18$ -deficient mice, which genetically lack iNKT cell population.<sup>25</sup> iNKT cells contribute to antitumor immunity and antimicrobial host responses in bacterial, parasitic, viral, and fungal infections in several models mostly through their IFN- $\gamma$ -mediated NK cell activation. In addition, iNKT cells have been shown to regulate autoimmunity such as T1D in NOD mice and EAE.<sup>26</sup> Meanwhile, we have demonstrated that iNKT cells were involved in the pathogenesis of arthritis by augmenting autoantibody-mediated inflammation and inflammatory immune response against type II collagen.<sup>27</sup> In addition, iNKT cells also participate in pathologic processes such as oxazolone colitis,<sup>28</sup> maternal intolerance of the fetus,<sup>29</sup> and formation of atherosclerotic plaque.<sup>30,31</sup> Therefore, iNKT cells are a double-edged sword, which enhance or suppress disease course depending on the immune condition of host. Interestingly, recent studies demonstrated the potent pathogenic roles of iNKT cells in the development of bronchial asthma.

iNKT cells are pre-activated *in vivo* by continuously encountering with endogenous lipid antigen(s) such



**Fig. 2** Two possible pathways for iNKT cell-dependent airway inflammation. See text for details.

as lysosomal glycosphingolipid, isoglobotrihexosylceramide (iGb3).<sup>32</sup> iNKT cells are prepared for forthcoming rapid production of regulatory cytokines by environmental trigger such as IL-12 and Toll-like receptor (TLR) ligand, direct recognition of exogenous glycolipid antigens, and antigen-specific activation of conventional T cell to control entire immune responses. Considering ceaseless encounter of iNKT cells with endogenous glycolipid antigen(s) to maintain pre-activated status of iNKT cells, one should consider that local infection or inflammation caused by irrelevant immune activation may influence iNKT cell responses, resulted in unexpectedly skewed cytokine production by these cells. Regarding local infection, a couple of microorganism-derived exogenous iNKT cell agonist has been reported to date.

$\alpha$ -glucuronosylceramide (GSL-1) and  $\alpha$ -galacturonosyl ceramide (GSL-1') derived from *Sphingomonas* species of  $\alpha$ -proteobacteria are shown to stimulate murine and human iNKT cells in a CD1d-dependent manner.<sup>33,34</sup> More recently, iNKT cells were demonstrated to exert protective activity against pathogenic *Borrelia* species of *Spirochaetaceae* family by recognizing bacterial cell wall-derived  $\alpha$ -galactosyl-diacylglycerolipids.<sup>35</sup> In addition to the recognition of microorganism-derived exogenous glycolipids, activation of TLR expressed on dendritic cells after bacterial infection results in massive IL-12 production and local inflammation, leading to altered behavior of iNKT cells. Weak responses to putative self antigens could be amplified by IL-12 produced by

dendritic cells (DCs) in response to microbial products, leading to augmented IFN- $\gamma$  secretion.<sup>36</sup> In fact, we have also clearly demonstrated that pattern of cytokine production by iNKT cells were greatly affected by local or systemic administration of TLR agonists.<sup>37</sup> Therefore, activation of iNKT cells is achieved through either direct recognition of specific antigens derived from infectious microorganisms or indirect augmentation of iNKT cells responses to endogenous antigens under specific (*i.e.* IL-12-rich) condition (Fig. 2).

### **INVOLVEMENT OF INKT CELLS IN THE DEVELOPMENT OF ALLERGIC RESONSES**

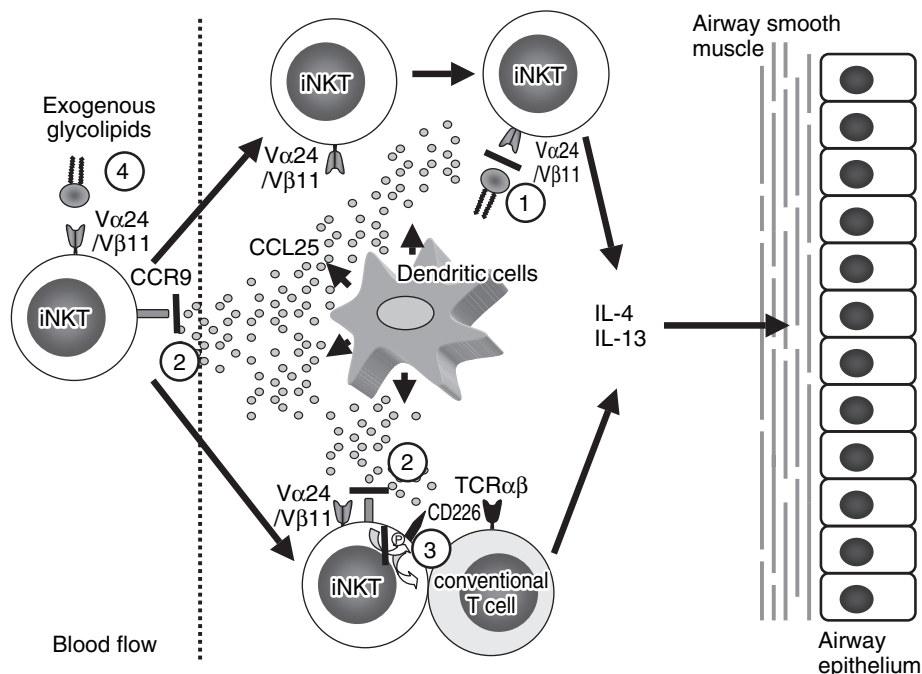
Given the outstanding ability to secrete a large amount of regulatory cytokines in exogenous antigen-dependent or -independent manner, versatile iNKT cells has been shown to be involved in diverse immune responses and immunological disorders. Relevance of iNKT cells on the development of allergic response was originally demonstrated almost a decade ago employing iNKT cell-deficient  $\beta 2$  microglobulin ( $\beta 2m$ ) knockout mice, in which NK1.1+ T cells were shown to be essential not only for the induction of IL-4-producing helper T cells, but also for IL-4-dependent class switching to immunoglobulin E in response to injection of anti-IgD antibody.<sup>38</sup> However, following studies did not always support the involvement of iNKT cell in IgE production.<sup>39-42</sup> CD1-deficient mice or J $\alpha$ 18-deficient mice lacking iNKT cells could mount a typical Th2 response to produce

IgE after immunization with anti-IgD antibody. Furthermore, even  $\beta 2m$ -deficient mice were shown to develop functional type 2 immune responses by immunization with ovalubmin (OVA), infection with *Leishmania major* or *Nippostrongylus brasiliensis*. These paradoxical results may reflect intrinsic complexity of the development of allergic responses where a diverse subset of immune cells could be primary IL-4 producer depending on different antigens, route of administration, and so on. In this connection, relatively common phospholipids such as phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) extracted from cypress grains induce proliferation of T cells derived from cypress-sensitive subjects.<sup>43</sup> Even though recognition of phospholipids involved CD4+ TCR $\alpha\beta$ + T cells that occur in a CD1a/d-dependent manner, V $\alpha$ 24+ human iNKT cells were rare in phospholipid-specific T cell clones in contrast to rather strong reactivity of murine V $\alpha$ 14 iNKT cell hybridomas to purified PE, phosphatidylglycerol (PG), and phosphatidyl inositol (PI). Interestingly, these T cell clones specifically respond to phospholipids derived from cypress pollen, but not phosphatidyl choline from brain, liver, or egg. Furthermore, allergic subjects displayed circulating antigen-specific IgE, cutaneous weal and flare reactions to phospholipids that may not require CD161+ NKT cells. Although it is clear that iNKT cells are not required for all immune responses leading to IgE production, further scrutiny will dissolve the conflicting evidence argued above for the involvement of iNKT cells on IgE responses *in vivo*.

Although the systemic allergic responses were too complex to clearly determine the involvement of iNKT cells, the development of OVA-induced allergic airway inflammation was shown to be impaired in the absence of iNKT cells in either J $\alpha$ 18-deficient or in CD1d-deficient mice.<sup>44</sup> The lack of AHR in these mice was associated with reduction of airway eosinophilia as indicated by a reduction of eosinophils in BAL fluid, reduction of OVA-specific IgE in their sera, and reduction of IL-4 and IL-13 production from their bronchial lymph node T cells. Interestingly, number of iNKT cells in the lungs of wild type mice was shown to be increased on airway challenge with OVA and these pulmonary iNKT cells were capable of producing IL-4 and IL-13 predominantly. Adoptive transfer of IL-4/IL-13-producing iNKT cells could restore the impaired AHR, demonstrating the essential roles of these cytokines produced by iNKT cells in the development of asthma. Furthermore, airway eosinophilia and mucus overproduction induced by ragweed were significantly reduced in CD1-deficient mice and the phenotype of these mice was well correlated with significantly lower IL-4 production and lower eotaxin responses in the airways.<sup>45</sup> Furthermore, activation of pulmonary iNKT cells by intranasal administration of  $\alpha$ GC or *Sphingomonas*-derived bacterial glycolipid is

sufficient for the development of AHR, eosinophilic infiltration in the absence of conventional CD4+ T cells and adaptive immunity.<sup>46</sup> MHC class II-deficient mice, which lack conventional CD4+ T cells, showed exacerbation of AHR when intranasally challenged with  $\alpha$ GC, indicating that iNKT cells are not only a modifier of AHR by producing cytokines to influence adaptive immunity, but are also critical effector cells for the development of AHR. These results clearly describe the role of iNKT cells in the pathogenesis of allergic asthma in experimental murine models.

As one could easily anticipate from the phenotypic similarity between murine and human iNKT cells, two papers suggesting the involvement of iNKT cells in the development of asthma have been published recently. Firstly, V $\alpha$ 24 iNKT cells derived from peripheral blood of patients with allergic asthma express CCR9 at high frequency.<sup>47</sup> CCR9 is commonly expressed on mucosa-homing lymphocytes concomitant with expression of  $\alpha 4\beta 7$ , which promotes adhesion to MAdCAM-1 on mucosal vessels. Massive accumulation of CCR9<sup>high</sup> iNKT cells in bronchial mucosa occurs in asthma patients and the numbers of CCR9+ iNKT cells from the peripheral blood of patients with asthma decreased either when they are asymptomatic or when steroid treatment is effective. CCR9 ligand CCL25 is shown to promote the recruitment of CCR9-expressing iNKT cells into the lung to develop allergic asthma. CCL25/CCR9 interaction induces phosphorylation of CD226 (DNAM-1) overexpressed on iNKT cells in asthma patients. CD226 engagement is required for iNKT cells to induce Th2 bias of conventional T cells because iNKT cell function for Th2 skewing is impaired by inhibition of CD226. Secondary, Akbari *et al.* reported that more than 60% of CD3+ or CD4+ T cells from patients with moderate-to-severe persistent asthma were the invariant T cell receptor-bearing V $\alpha$ 24 iNKT cells detected by  $\alpha$ GC-loaded CD1d tetramer.<sup>48</sup> iNKT cell derived from airway produced IL-4 and IL-13, but not IFN- $\gamma$  upon stimulation with  $\alpha$ GC. Massive infiltration of iNKT cells is specific for asthma because the most CD4+ T cells found in the lungs of patients with sarcoidosis were conventional T cells. Detailed examination of bronchial biopsy specimens from the patients with asthma revealed that nearly all the lamina propria lymphocytes expressed CD4 and V $\alpha$ 24 invariant TCR. Intriguingly, the vast majority of the iNKT cells coexpressed CD4+ in the BAL fluid of patient with asthma, whereas approximately 50% of the iNKT cells in peripheral blood were double-negative (CD4- / CD8-). CD4+ iNKT cells producing Th2 cytokines may be selectively recruited into the lungs of patients with bronchial asthma through the selective induction of CCR9 in circulating iNKT cells. Taken together, these two reports provided strong evidences to suggest the involvement of iNKT cells in the pathogenesis of allergic asthma not only in experimental



**Fig. 3** Implication for the possible target for treatment of asthma. 1. Prevention of activation of iNKT cells by blocking Vα24. 2. Prevention of entry of the activated pulmonary iNKT cells into airway mucosa by inhibiting CCL25 or CCR9. 3. Prevention of Th2 polarization of conventional CD4+ T cells by inhibiting cell-cell contact-driven phosphorylation of CD226. 4. Stimulation of iNKT cells with synthetic glycolipid ligand(s) that inhibit Th2-skewing iNKT cell function

murine model, but also in human diseases. CD4+ iNKT cells and conventional CD4+ T cells have a number of shared phenotypes somewhat hard to discriminate each other. Even though antigen-specific T cell response is essential for initiation of asthmatic diseases, iNKT cells might have been underestimated in a number of past studies to show that conventional Th2 cells play a predominant role in every step of asthmatic diseases. For example, human iNKT clones cultured with irradiated antigen-presenting cells in the presence of IL-2 exert selective IL-5 production in the absence of exogenous antigens, implying direct roles of iNKT cell on eosinophilia (unpublished observation). Therefore, the involvement of iNKT cells in the pathogenesis or enhancement of asthma should be re-evaluated.

### **THERAPEUTIC POTENTIALS OF GLYCOLIPID LIGANDS FOR INKT CELLS FOR ALLERGIC ASTHMA**

The findings that iNKT cells are involved in the development of allergic asthma called people's attention to apply glycolipid ligands for modification of iNKT cell function and possible intervention of allergic diseases. In fact, there are many reports describing the effects of αGC on murine experimental allergic asthma model. Some of them provide the data to sup-

port the application of αGC for glycolipid therapy of asthma but the others do not. A single intravenous or intranasal injection of αGC just before the first antigen challenge of sensitized mice was shown to abrogate elicitation of AHR, airway eosinophilia, IL-4 and IL-5 level in BAL fluid and the serum level of OVA-specific IgE.<sup>49</sup> IL-13 production and goblet cell hyperplasia was inhibited as well by an intraperitoneal injection of αGC.<sup>50</sup> The therapeutic effects of αGC are associated with a shift of cytokine response from Th2 to Th1 in BAL fluid and IFN-γ has been shown to play a pivotal role for the effects of αGC. Therefore, αGC injection at the time of antigen challenge could protect host from the development of typical asthmatic symptoms. However, the development of AHR by intranasal administration of αGC might be a concern as a side effect. Repeated exposure of mice to αGC has been shown to induce Th2 polarization by reducing levels of IFN-γ and the protocol of continuous administration of αGC during sensitization with antigen actually increased antigen-specific IgE levels in serum and worsened eosinophilic infiltration into lung.<sup>51</sup> In addition, intranasal coadministration of allergen and αGC induced AHR, eosinophilic infiltration, elevation of IgE level, and production of Th2 cytokines.<sup>52</sup> Considering the outstanding ability of iNKT cells for the production of a variety of cytokines, iNKT cells are a

double-edged sword, which enhance or suppress disease course depending on the immune condition of host and application of glycolipid ligands for iNKT cells on therapeutic intervention of asthma should be given a particularly careful attention on potential risk of the compound.

We have been screening  $\alpha$ -GC analogues to select glycolipid(s) other than  $\alpha$ GC that have potent therapeutic effects on murine experimental asthma model hopefully without exerting side effects. We have identified a novel synthetic glycolipid that inhibit Th2 cytokine production (IL-4, IL-5, IL-13), eosinophilic infiltration goblet cell hyperplasia when administrated before the antigen challenge. The effector mechanism of this novel glycolipid is not clear yet and detailed analysis is now under investigation. As growing evidences have supported the involvement of iNKT cells in allergic asthma, a search for glycolipid antigens for iNKT cells to manipulate the pleiotropic functions will provide a novel and unique therapeutic approach for allergic asthma.

## CONCLUSIONS

In this review, we overviewed current understanding of the pathophysiologic roles of iNKT cells in asthma. We also discussed on possible therapeutic approaches for asthma employing glycolipid ligand for iNKT cells (Fig. 3). Over the past several years, we have learned more about specific mechanisms causing allergic inflammation such as TSLP and IL-25, which has led to novel strategies for the treatment of asthmatic diseases. The activation of innate immune response for the inhibition of allergy is of particular interest. For example, CpG oligodeoxynucleotides, a TLR9 ligand, inhibit Th2 cytokine production. Interestingly, intestinal commensal flora might provide anti-allergic effect by signaling through TLR4. TLR4-deficient mice were more susceptible to food allergy and stimulation by intestinal microbes influences susceptibility to food allergy.<sup>53</sup> Moreover, administration of antibiotics converted allergy-resistant wild type mice to allergy-susceptible. These data suggest that elimination of commensal bacteria prevents development of oral tolerance to allergens, and enhances allergic sensitization. We have recently reported the protective roles of another invariant NKT ( $V\alpha 19$  iNKT) cells for the development of EAE.<sup>54</sup>  $V\alpha 19$  iNKT cells are functionally overlapping with mucosa-associated invariant T (MAIT) cells.<sup>55,56</sup> On the analogy of functional involvement of iNKT ( $V\alpha 14$  in mouse, or  $V\alpha 24$  in human) cells, mucosa-associated second iNKT subset might have functional relevance on the development of asthma. Taken together, functional manipulation of iNKT cells by specific glycolipid ligands will provide a novel approach for the development of new therapeutic strategy for allergy and asthma.

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