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■ Review article ■

Natural killer T cell and pathophysiology of asthma

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= Abstract =

Natural killer T (NKT) cell is a special type of T lymphocytes that has both receptor of natural killer (NK) cell (NK1.1, CD161c) and T cell (TCR) and express a conserved or invariant T cell receptor called Vα14Jα18 in mice or Va24 in humans. Invariant NKT (iNKT) cell recognizes lipid antigen presented by CD1d molecules. Marine-sponge-derived glycolipid, α-galactosylceremide (α-GalCer), binds CD1d at the cell surface of antigen-presenting cells and is presented to iNKT cells. Within hours, iNKT cells become activated and start to secrete Interleukin-4 and interferon-γ. NKT cell prevents autoimmune diseases, such as type 1 diabetes, experimental allergic encephalomyelitis, systemic lupus erythematous, inflammatory colitis, and Graves' thyroiditis, by activation with α-GalCer. In addition, NKT cell is associated with infectious diseases by mycobacteria, leshmania, and virus. Moreover NKT cell is associated with asthma, especially CD4+ iNKT cells. In this review, I will discuss the characteristics of NKT cell and the association with inflammatory diseases, especially asthma. (Korean J Pediatr 2010;53:136-145)

Key Words : NKT cell, Asthma, CD1d molecule, α-GalCer

Introduction

Allergic disease has become the most common chronic illness among children and the prevalence of allergic disease is projected to increase in years to come, related to factors including a rise in air pollution, changing pattern of food habit and environment[1].

Asthma has several phenotypes characterized by symptoms of recurrent wheezing, coughing, breathlessness and chest tightness due to airway hyperreactivity (AHR) to stimuli. The most common form of asthma is allergic asthma, and there are many other forms (e.g., infection-induced, exercise-induced, aspirin-associated), reflecting multiple different pathogenic mechanisms and indicating that asthma represents a collection of heterogeneous syndromes[2]. Asthma is chronic inflammation of the airways and related to the presence of mast cells, eosinophils, T cells, B cells, basophils, and cytokines interleukin (IL)-4, IL-5, IL-13, and IL-17. The conventional, major histocompatibility complex (MHC)-class II-restricted $CD4^+$ T cells have been thought to have a crucial and obligatory role in the pathogenesis of bronchial asthma[3]. Some investigators have shown that $CD4^+$ invariant natural killer T(iNKT) cells are required for the development of allergen-induced AHR in mouse models of asthma[4, 5]. Recent studies propose that $CD4^+$ $_i$NKT cells have as important a role as conventional $CD4^+$ T cells in the pathogenesis of asthma. $CD4^+$ NKT cells that express an invariant T-cell receptor (TCR) and that produce T helper (TH)2 cytokines might therefore look similar to conventional $CD4^+$ T cells and function in a similar manner. It is considered that these TH_2-like $_i$NKT cells have a crucial role in regulating the development of asthma[6].

Allergen-induced AHR fails to develop in $CD1d^{-/-}$ mice, which lack the restriction element of NKT cells and therefore lack NKT cells, and fails to develop in $J18^{-/-}$ mice[4, 7]. Moreover, recent studies have shown that other forms of AHR induced by exposure to ozone or with pulmonary virus infection also require the presence of $_i$NKT cells[8, 9]. Type I $_i$NKT cells are increased in lungs of patients with severe asthma compared with that in normal individuals. NKT cells have an important role in immune responses and pathophysiology in variable diseases, however consist of less than 1 % of total lymphocytes[6]. NKT cells can secrete huge amounts of cytokines such as in-

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terferon-γ, IL-4, and IL-13 within several hours after activation. Especially NKT cells can promote TH1 response and TH_2 response, either[10]. This article describe the characteristics of NKT cells and association with asthma, especially $CD4^+$ iNKT cells.

History

In 1986, the Vα14 Jα18 suppressor T cell clones was established and Budd et al. reported the subset of αβ-T cell receptor $(TCR)^+$ T cells and lacked expression of CD4 and CD8 accessory molecules in 1987[11]. NK 1.1^+ T cell identified by Skyes et al in 1990[12]. Zlotnik et al reported that cytokines production by mature and immature $CD4^-$ $CD8^-$(double negative; DN)-αβ-$TCR^{(+)}$ T cells in 1992[13]. In 1993 $NK1.1^+CD4^+$ cells were also found to be a potent source of immunoregulatory cytokines while $NK1.1^-$ $CD4^+$ thymocytes were not a potent source of cytokines[14]. NKT cells are reactive to the MHC class-I-like molecule 'CD1d' and TCR Vβ-chain usage towards Vβ8.2, most $NK1.1^+$ T cells were found to use an invariant TCR α-chain consisting of Vα14-Jα18 in 1994[15]. The term 'NKT cells' was defined as a subset of mouse T cells that have NK1.1 marker (CD161c), first mentioned by Makino et al. in 1995[16].

CD1d molecules present hydrophobic/lipid antigens, and a model CD1d-reactive glycolipid antigen- the marine-sponge-derived agent (2S, 3S, 4R)-1-O-(α-D-galactopyranosyl)- N-hexacosanoyl- 2-amino-1,3,4-octadecanetriol), commonly referred to as α-galactosylceramide (α-GalCer)- was identified as a potent stimulatory factor for NKT cells[17]. α-GalCer was discovered by the Kirin Pharmaceutical Research Corporation as part of a screen for substances that could prevent the metastasis of transplanted tumors to the livers of mice. It was found originally in an extract of the marine sponge *Agelas mauritanius*, and later, a synthetic analogue of this compound was developed for experimental studies and clinical trials[18]. There are several kinds of self and microbial glycosphingolipid ligands (GSL) of NKT cells. Marine sponge αGalCer (KRN7000) with carbon atom number assignments on sphingosine, acyl, and carbohydrate, *Sphingomonas* GSL-1 through GSL-4, mammalian isoglobotrihexosylceramide (iGb3), or Galα1,3 Galβ1,4Glcβ1,1Cer, and αGalactosyldiacylglycerols from *Borrelia burgdorferi*. The proximal glucose of the mammalian glycosphingolipid has a β-anomeric linkage to ceramide, in contrast with the α-branched galactose of αGalCer or glucuronyl of *Sphingomonas* GSLs. These lipids potently activate CD1d restricted NKT cells that express the semi-invariant Vα14-Jα18 TCR in mice and the Vα24-Jα18 equivalent cells in humans[8].

Types & development of NKT cell

NKT cells are heterogeneous group. $NK1.1^+$ classical (type I) NKT cells that either express or do not express the invariant Vα14-Jα18 (Vα24-Jα18 in humans) TCR and/or NK1.1 (CD161 in humans) have been identified, but not all $NK1.1^+$ T cells are type I NKT cells. They include other CD1d-dependent (type II) NKT cells, as well as CD1d-independent (Type III NKT-like cells) T cells, which might vary widely in function. The most reliable way to detect type I NKT cells in mice and humans is by using CD1d tetramers loaded with α-GalCer[19].

Category I is the type I iNKT cell group. These cells have a rearrangement of the variable region Vβ8.2, Vβ7, and Vβ2 (Vβ11 in humans) TCR and/or $CD4^+$ and DN have been identified, but $CD8^+$ identified also in humans. Recently, iNKT cell has new subset identified by express or do not express the IL17RB. Different functional phenotypes have also been observed with $Vα24^+$ NKT cells defined by the presence or absence of CD4 expression in human peripheral blood. The $CD4^+$ NKT-cell subset produced higher levels of IL-4, IL-13, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-2 than the CD4-NKT cells, whereas both populations produced IFN-γ and TNF at high levels. Category II is type II non-classical NKT cell group. These cells in mice and humans have also CD1d dependent properties, and include a subset of NK1.1-cells that exist in both the thymus and the periphery of mice. These cells have been less well characterized, mainly because they cannot be identified using α-GalCer-loaded CD1d tetramers, and therefore the exact prevalence of these cells and the extent to which they express NK1.1 is not precisely known. TCR of these cells express are more diverse either $CD4^+$ or DN CD1d- reactive cells make an almost undetectable contribution to the repertoire of mouse $CD8^+$ T cells, but $CD8^+$ identified also in humans. Category III is type III NKT-like cell group. These cells express $NK1.1^+$ but not CD1d dependent cells that have diverse TCRs and can be $CD8^+$, $CD4^+$ or DN. These $NK1.1^+$ T cells are enriched in the spleen and bone marrow compared with

the thymus and liver. Expression of NK1.1 is a marker for the activation of conventional T cells, such as those that are stimulated by viral antigens. In humans, the frequency of $CD161^+$ $TCR\beta^+$ T cells is much higher than the frequency of Vα24i T cells. Category IV is also type III NKT-like cell group. These cells are defined by the expression of CD49B (α2-integrin), which is recognized by the DX5 antibody. There is very little overlap between this population and the Vα14i T cells. $CD49B^+$ cells might have specialized roles in the antigen-specific suppression of immune responses in irradiated skin and in the suppression of autoimmune diabetes (Table 1)[19, 20].

A developmental pathway for NKT cells. Classical (type I) natural killer T (NKT) cells and conventional $CD8^+$ and $CD4^+$ T cells diverge from a common precursor ($CD4^+$ $CD8^+$ double-positive, DP) thymocytes. Random TCR-gene rearrangement leading to expression of Vα14-Jα18 in conjunction with either Vβ8.2, Vβ7 or Vβ2 leads to the CD1d-dependent selection and branching of the NKT-cell lineage. Upon expression of their canonical TCRα chain, which requires survival signals induced by RORγt, NKT cell precursors interact with endogenous agonist ligands such as iGb3, presented by CD1d expressed on other DP thymocytes in the cortex. NK1.1 expression is a downstream event in the maturation of NKT cells, and most NKT cells migrate from the thymus to the periphery before this stage, although some $NK1.1^+$ NKT cells can also emigrate. Mature $NK1.1^+$ NKT cells undergo clonal expansion in the periphery after activation. The transcription factor T-bet is required for induction of the IL-15 receptor β chain and survival at the late-memory and NK1.1 stages.

Thymic emigrant NKT cells in the periphery did not express NK1.1, which implied that maturation to the $NK1.1^+$ stage could take place in the thymus or in the periphery. Immature $NK1.1^-$NKT cell populations in the thymus can be subdivided on the basis of CD24, CD44 and DX5 expression, thereby identifying at least four phenotypically distinct stages within the broad $NK1.1^-$ immature phase: $NK1.1^-$ $CD24^+CD44^{low}DX5^{low}$ stage 1 cells; $NK1.1^-$ $CD24^{low}CD44^{low}$ $DX5^{low}$ stage 2 cells; $NK1.1^-CD24^{low}CD44^{hi}DX5^{low}$ stage 3 cells; and $NK1.1^-$ $CD24^{low}CD44^{hi}DX5^{hi}$ stage 4 cells. Stage 1 (CD24+) cells are present at a very low frequency (approximately 1 in 10^6 thymocytes) and, in contrast to other $NK1.1^-$NKT cells, they are small and apparently non-dividing, suggesting that the extensive post-selection expansion follows this stage. The precursor-progeny relationship between stages 1 to 4 has not been formally demonstrated, but the pathway fits well with ontogeny data from independent groups. The implication is that cells at stage 4 are the immediate precursors of the mature $NK1.1^+$ NKT cells (Fig. 1)[19, 21].

Characteristics of NKT cell

NKT cell show different pattern on secretion of cytokines, according to the time-dependent manner after activation. Kaer described that "soon after administration of α-GalCer to mice, this reagent binds CD1d at the cell surface of antigen-presenting cells (APCs) and is presented to iNKT cells. Within hours, iNKT cells become activated

Table 1. Classification of NKT cells

Type category	Type I iNKT cells	Type II NKT cells	Type III NKT-like cells	
	I	II	III	IV
Ag pesenting molecule	CD1d	CD1d	MHC I, others	MHC I, MHC II
Reactivity	α GalCer	ND	Self-agonist	ND
TCR α chain	Vα14-Jα18 (mice) Vα24-Jα18 (humans)	Semi-diverse, but some Vα3.2-Jα9 (mice)	Diverse	Diverse
TCR β chain	Vβ8.2,Vβ7 and Vβ2 (mice) Vβ11 (humans)	Semi-diverse, but some Vβ8 (mice)	Diverse	Diverse
Subsets	$CD4^+$ and DN (mice) $CD4^+$, $CD8^+$ and DN (humans) $IL17RB^{+/-}$ (mice)	$CD4^+$ and DN (mice)	$CD4^+$,$CD8^+$ or DN	$CD4^+$ or $CD8^+$ CD49B
NK receptors	$DX5^-$ $NK1.1^+$ (mostly, resting mature) $^{-/low}$ (immature or post-activation)	$DX5^{(?)}$ $NK1.1^{+/-}$	$DX5^{+/-}$ $NK1.1^+$	$DX5^+$ $NK1.1^{+/-}$

Abbreviations : ND, not determined; modified from Godfrey et al[19] and Kronenberg et al[20]

and start to secrete IL-4 and IFN-γ. αGalCer-activated iNKT cells also rapidly downregulate cell-surface-expressed TCR and NK1.1, rendering these cells undetectable with flow-cytometric reagents that bind these markers. TCR expression levels return to almost normal at -24 hours (hr) after injection of α-GalCer. iNKT cells then rapidly proliferate, clonally expanding 10- to 15-fold in the spleen and less extensively in other organs. Most iNKT cells subsequently die, to maintain homeostatic numbers. The cell-surface expression levels of NK1.1 remain suppressed for an extended period. Early (2 hr) during the response to α-GalCer, iNKT cells mainly produce IL-4. At 24 hr, these cells mainly produce IFN-γ, and at 3 days after injection of α-GalCer, when iNKT-cell numbers are maximal, these cells produce few cytokines"[10].

αGalCer. This compound binds to CD1d, and the αGalCer-CD1d complex is recognized by mouse Vα14i and human V α24i T-cell receptors (TCRs). αGalCer is a glycosphingolipid, a chemical category that encompasses abundant glycolipids in the body, including the gangliosides[22].

Vα14 iNKT cells are found at the highest frequency in liver (>10% of liver lymphocytes), but are present at lower frequencies (typically <1%) in thymus, bone marrow, spleen, and blood, and at even lower frequencies in lymph nodes. Kronenberg et al. described that "a different developmental pathway with positive selection mediated by $CD1d^{+}$ double positive thymocytes rather than cortical epithelial cells. Human Vα24 iNKT cells tend to be much less abundant than their Vα14 iNKT cell counterparts, and they are not highly enriched in the liver, although their frequency in peripheral blood mononuclear cells can vary over two orders of magnitude in healthy individuals, and they constitute only a small percentage of the cells that express an αβ TCR and NK receptors such as CD161"[20].

iNKT cells are stimulated by CD1d-expressing APCs that are loaded with αGalCer. Bendelac et al. described that "iNKT cells receive stimulatory signals from APCs (through IL-12) and, in return, the iNKT cells stimulate APCs by secretion of IFN-γ. After stimulation, iNKT cells can exert effector functions (for example, cytotoxicity through FAS-AS-ligand and perforin/granzyme pathways, and immunoregulatory functions (for example, secretion of the cytokines IL-4, TGF-β and IFN-γ). During myeloid DC maturation, expression of CD1d is upregulated and activates CD1d-restricted T cells, which can promote activation-induced lysis or the differentiation of DCs"[17].

Dual recognition of self and microbial glycosphingolipids during microbial infections. Bendelac et al. described that "infection by Gram-negative, LPS-negative *Sphingomonas* induces direct activation of NKT cells through recognition of microbial cell wall α-glycuronylceramide. Infection by Gram-negative, LPS-positive *Salmonella* activates TLR4 through LPS and induces IL-12, revealing constitutive autoreactive recognition of iGb3 through the secretion of

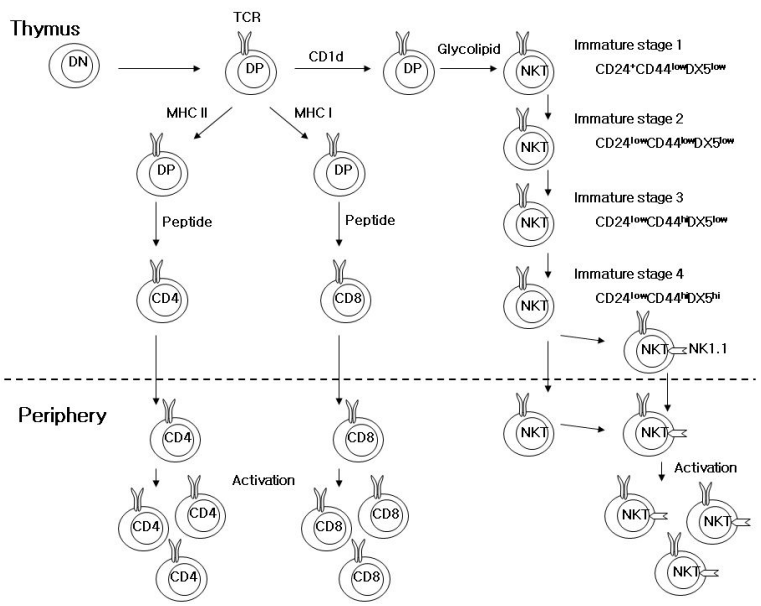

Fig. 1. Development of NKT cells. Modified from Godfrey et al[19, 21].

IFN-γ (indirect microbial recognition) In addition, NKT cell is associate with infectious disease, such as mycobacteria, leshmania, and viral infection"[8].

iNKT cells have been implicated in several models of tumor rejection, although the precise details vary. Wilson et al. reported that iNKT cells encounter a dendritic cell (DC) expressing CD1d complexes with either exogenous α-GalCer or an endogenous activating ligand, and they are activated to secrete cytokines, mainly IFN-γ and upregulate expression of cell-surface co-stimulatory molecules. The sequential production of IFN-γ by both iNKT cells and NK cells has been shown to be required for the antimetastatic activity of α-GalCer. Appropriately activated DCs can stimulate NK cells and tumor-specific cytotoxic T lymphocytes (CTLs). iNKT cells can also inhibit tumor surveillance in an IL-13- and STAT6-dependent manner"[23].

Autoimmune disease should be associated with NKT cell. Kaer described that a reduced frequency of iNKT cells is associated with autoimmune disease. First, in autoimmune diabetes, iNKT-cell responses to activation signals are impaired. Transfer or activation of the candidate cell should protect against disease. Transfer of highly enriched iNKT-cell populations protects mice from developing diabetes. Second, NKT cell associated with rheumatoid arthritis. FCRIII engagement provides activating signals to NKT cells in antibody-induced joint inflammation and NKT cells promote antibody-induced joint inflammation by suppressing TGFβ1 production. NKT cell prevents other autoimmune disease, such as experimental allergic encephalomyelitis (EAE), systemic lupus erythematous (SLE), inflammatory colitis, Graves' thyroiditis, by activation with αGalCer."[10].

Hypersensitivity pneumonitis (HP) is a kind of interstitial lung diseases (ILD) induced by sensitization of organic dust or mold was inhaled through airway, repeatedly. Hwang et al reported that "IL-4-secreting NKT cells play a protective role in Saccharopolyspora rectivirgula (SR)-induced HP by suppressing IFN-γ-producing neutrophils, which induce the activation and proliferation of $CD8^+$ T cells in the lung."[24]. Korosec et al reported that a major subset of NKT cells in the BALF of patients with hypersensitivity pneumonitis was a $CD8^+CD56^+$ population that did not express the invariant TCR and significantly higher frequencies of pulmonary NKT cells in patients with hypersensitivity pneumonitis in comparison to the other study patients with ILD[25].

iNKT-cell is associated with atopic eczema (AE). The inflammation of AE is orchestrated not only by T cells predominantly but also B cells, eosinophils and dendritic cells and commensal yeast *Malassezia* can activates human DC to produce IL-18, an innate cytokine that is elevated in serum of AE patients. IL-18 was a potent activator of human iNKT-cells and promoted a proinflammatory CD1d-dependent response, even in the absence of exogenous ligands. Linda et al. reported that IL-18-mediated activation and subsequent dysregulation of the CD1d-restricted iNKT cells plays a role in the pathogenesis of human AE. Chronic activation via IL-18 on the other hand was inhibitory and skewed the iNKT-cell pool by selectively suppressing iNKT-cells and also shown on AE patients where the proportion of $CD4^+$ iNKT-cells was reduced in peripheral blood and coincided with elevated plasma levels of IL-18 [26]. Simon et al. reported that NKT cells have been detected in small numbers in the majority of atopic eczema specimens as well as in atopy patch test (APT) reactions, allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD). In AE, the proportion of NKT cells among $CD3^+$ cells was approximately 5%. NKT cells expressed both IFNγ and IL-4 in AE, APT and ACD but predominantly IFNγ in ICD. Natural killer T cells are part of the inflammatory infiltrate of AE as well as APT, ACD and ICD, suggesting a pathogenic role of NKT cells in eczematous skin disorders. The pattern of IFN-γ and IL-4 cytokine expression by NKT cells varied depending on the type of eczematous disease[27].

Pulmonary fibrosis is a progressive illness characterized by interstitial fibrosis. Although the precise mechanism for pulmonary fibrosis is not completely understood, an immune response involving IFN-γappears to play a role. NKT cell produce IFN-γ and IL-4 on activation, in bleomycin-induced pulmonary fibrosis. The transforming growth factor (TGF)-β1 levels were higher in the lung after injecting bleomycin, and blockade of TGF-β1 by neutralizing monoclonal antibody attenuated the pulmonary fibrosis in $CD1d^{-/-}$ mice. Kim et al reported that IFN-γ-producing NKT cells play a novel anti-fibrotic role in pulmonary fibrosis by regulating TGF-β1 production. And in vitro assay demonstrated that IFN-γ was involved in suppressing TGF-β1 production in cells collected from bronchoalveolar lavage[28].

NKT-cell in mouse model of chronic obstructive pulmonary disease (COPD). COPD is mediated by $CD4^+$ T

cells same as asthma. However, COPD is associated with CCL2, CXCL1, and CXCL8 that attract TH1 cells, monocytes that differentiate into macrophages and polymorphonuclear phagocytes (neutrophils). Kim et al, investigated how Sendai virus infection prompted chronic lung disease, and its symptoms evolved independently of adaptive lymphocytes but required interactions between CD4− NKT cells and macrophages. These interactions occurred via CD1d. IL−13 derived from NKT cells, acts on lung macrophages, stimulating them to produce more IL−13, resulting in an autocrine signaling loop involving IL−13 and its receptor[5]. Holtzman et al. analyzed a mouse model of lung disease that develops after respiratory viral infection. When the acute lung disease appears in this model (at 3 weeks after viral inoculation), it depends on an immune axis that is initiated by expression and activation of the high−affinity IgE receptor (FcεRI) on conventional lung dendritic cells (cDCs) to recruit IL−13−producing $CD4^+$ T cells to the lower airways. However, when the chronic lung disease develops fully (at 7 weeks after inoculation), it is driven instead by an innate immune axis that relies on iNKT cells that are programmed to activate macrophages to produce IL−13. This innate immune axis is also activated in the lungs of humans with severe asthma or COPD based on detection of increased numbers of iNKT cells and alternatively activated IL−13−producing macrophages in the lung"[29].

NKT cell and asthma

In animal asthma models, NKT cell deficient (CD1d−deficient) mice failed to develop allergen−induced AHR, airway eosinophilia as well as immunoglobulin E (IgE) and Th2 cytokine production after allergen challenge. This failure could be reversed by the adoptive transfer of NKT cells. Thus, the observation that NKT cells are present among pulmonary $CD4^+CD3^+$ T cells in asthma patients seemed logical, although the reported frequency of 60% was rather high[6]. These mouse studies provide a possible explanation for the observation that many patients with allergic rhinitis, who develop allergen sensitization and have allergen−specific TH2 cell and IgE responses, do not automatically develop asthma, a disease that in humans might also require the involvement of NKT cells.

The α−chain of CD1d associates with β2−microglobulin (β2m) to form the complete heterodimeric CD1d molecule. Allergen−induced AHR, however, develops in β2−microglobulin $(\beta2m)^{-/-}$ mice, which lack classical iNKT cells, suggesting that in some situations iNKT cells may be dispensable for the development of AHR. Mice deficient in β2m lack all MHC class I molecules, including CD1d, and are devoid of $CD8^+$ cells and iNKT cells. Koh et al. suggest that "a CD1d−restricted, NK1.1 noninvariant TCR NKT cell population is present in $\beta2^{-/-}$ and is responsible for the development of AHR but not for Th2 responses. Furthermore, treatment of $\beta2m^{-/-}$ with anti−CD1d mAb or anti− NK1.1 mAb unexpectedly abolished allergen−induced AHR. The CD1−restricted NKT cells in these mice, which failed to respond to αGalCer and which therefore were not classical type I iNKT cells, appear to represent an NKT cell subset restricted by a β2m−independent form of CD1d. These results indicate that, although classical type I iNKT cells are normally required for the development of AHR, under different circumstances other NKT cell subsets, including nonclassical NKT cells, may substitute for classical iNKT cells and induce AHR"[30].

$J\alpha18^{-/-}$mice, which lack the α−chain of the invariant TCR and therefore specifically lack type 1 iNKT cells[7]. Like the $CD1d^{-/-}$mice, the $J\alpha18^{-/-}$mice failed to develop AHR when allergen sensitized and challenged, and showed reduced airway eosinophilia. Adoptive transfer of purified wild−type iNKT cells into the $J\alpha18^{-/-}$mice prior to allergen challenge fully reconstituted airway inflammation and AHR, indicating that iNKTcells were specifically required for the development of AHR Production of IL−4 and IL−13 by the iNKT cells is evidentially required for the development of AHR, since adoptive transfer of iNKT cells from $IL-4^{-/-}IL-13^{-/-}$mice into $J\alpha18^{-/-}$mice failed to restore AHR[4].

Mice that lack the transcription factor T−bet, which have a profound deficiency in the ability to generate iNKT cells in the periphery due to a halt in terminal maturation, but despite this deficiency, T−bet−deficient mice develop spontaneous AHR and airway inflammation. Kim et al. investigated that AHR response in the T−bet−deficient mice after administration of αGalCer. In T−bet−deficient mice, spontaneous AHR as well as AHR induced with OVA or α GalCer were all eliminated by blocking CD1d, the restricting element of iNKT cells, using an anti−CD1d−blocking mAb. Although the number of the iNKT cells in T−bet−deficient mice was reduced compared with that in wild−type mice, the remaining iNKT cells produced primarily IL−4 and

IL-13, and only minimal amounts of IFN-γ. They suggested that the remaining immature iNKT cells in T-bet-deficient mice preferentially produce IL-4 and induce AHR, because immature iNKT cells express IL-4 but only small amounts of IFN-γ[31].

Diesel exhaust particles (DEP) have strong, selective Th2 adjuvant activity when inhaled with conventional antigens. Finkelman et al. used "a novel technique for measuring in vivo cytokine production to investigate possible mechanisms by which DEP might promote a Th2 response. Intraperitoneal injection of DEP stimulated IL-6 secretion, but failed to increase IL-4, IL-10, or TNF-α secretion, and decreased basal levels of IFN-γ. When injected with or before LPS, DEP had little effect on the LPS-induced TNF-α responses, but partially inhibited the LPS-induced IL-10 response and strongly inhibited the LPS-induced IFN-γ response. They suggest that DEP inhibit Toll-like receptor ligand-induced IFN-γ responses by interfering with cytokine signaling pathways that stimulate NK and NKT cells to produce IFN-γ. DEP may promote a Th2 response by stimulating production of inflammatory cytokines while simultaneously inhibiting production of IFN-γ, and raise the possibility that the same mechanisms contribute to the association between DEP exposure and asthma"[32].

Exposure to ozone, which is a major component of air pollution, induces a form of asthma that occurs in the absence of adaptive immunity. Although ozone-induced asthma is characterized by airway neutrophilia, and not eosinophilia, it is nevertheless associated with AHR, which is a cardinal feature of asthma. Pichavant et al. found that "repeated exposure of wild-type (WT) mice to ozone induced severe AHR associated with an increase in airway NKT cells, neutrophils, and macrophages. NKT cell-deficient (CD1d $^{-/-}$ and Jα18$^{-/-}$) mice failed to develop ozone-induced AHR. Further, treatment of WT mice with an anti-CD1d mAb blocked NKT cell activation and prevented ozone-induced AHR. Moreover, ozone-induced, but not allergen-induced, AHR was associated with NKT cells producing IL-17, and failed to occur in IL-17$^{-/-}$ mice nor in WT mice treated with anti-IL-17 mAb. Thus, ozone exposure induces AHR that requires the presence of NKT cells and IL-17 production. Because NKT cells are required for the development of two very disparate forms of AHR, ozone-induced AHR is associated with neutrophils and IL-17, whereas allergen-induced AHR is associated with eosinophils, but primarily with IL-4 and not IL-17, these results strongly suggest that NKT cells mediate a unifying pathogenic mechanism for several distinct forms of asthma, and represent a unique target for effective asthma therapy. Moreover, the role of iNKT cells in sensing oxidized lipids may explain the observation that obesity increases the risk of developing asthma in humans and in mice. Thus, oxidized lipids, which may develop readily in obese individuals or in obese mice, might serve as an important "antigen" that activates iNKT cells, leading to the development of AHR and atherosclerotic heart disease"[9].

IL-25 has been shown to induce Th2 responses and AHR in mice, but the mechanism of action is not understood and it is unclear which cells mediate this disease. Terashima et al. investigated "a novel subset of NKT cells that expresses the interleukin 17 receptor B (IL-17RB) for IL-25 (also known as IL-17E) and is essential for the induction of AHR. IL-17RB is preferentially expressed on a fraction of CD4$^+$ NKT cells but not on other splenic leukocyte populations tested. IL-17RB$^+$ CD4$^+$ NKT cells produce predominantly IL-13 and Th2 chemokines upon stimulation with IL-25 in vitro. IL-17RB$^+$ NKT cells were detected in the lung, and depletion of IL-17RB$^+$ NKT cells by IL-17RB-specific monoclonal antibodies or NKT cell-deficient Jα18$^{-/-}$ mice failed to develop IL-25-dependent AHR. Cell transfer of IL-17RB$^+$ but not IL-17 RB$^-$ NKT cells into Jα18$^{-/-}$ mice also successfully reconstituted AHR induction. They suggest that IL-17RB$^+$ CD4$^+$ NKT cells play a crucial role in the pathogenesis of asthma[33]. Stock et al. reported that the receptor for IL-25, IL-17RB, is highly expressed on a subset of naive and activated CD4$^+$ iNKT cells, but not on activated T cells. IL-17RB$^+$ iNKT cells produced large amounts of Th2 cytokines that were substantially increased by IL-25 stimulation. Furthermore, IL-17RB$^+$ iNKT cells were capable of restoring AHR in iNKT cell-deficient mice, whereas IL-17RB-iNKT cells failed to reconstitute AHR and lung inflammation. Finally, IL-17RB$^+$ iNKT cells were detected in the lungs of wild-type mice, and induction of AHR by intranasal administration of IL-25 was significantly impaired in iNKT cell-deficient mice. They suggest a critical role for iNKT cells in IL-25-mediated AHR. These results may lead to novel therapeutic approaches to target IL-17RB iNKT cells for the treatment of allergic asthma[34].

PD-L1 and PD-L2 modulate airway inflammation and iNKT-cell-dependent AHR in opposing directions. Interactions of the inhibitory receptor programmed death-1 (PD-

1) with its ligands, programmed death ligand (PD-L)1 and PD-L2, regulate T-cell activation and tolerance. Akabari et al. investigated the role of PD-L1 and PD-L2 in regulating iNKT-cell-mediated AHR in a murine model of asthma. The severity of AHR and airway inflammation is significantly greater in PD-L2$^{-/-}$ mice compared with wild-type mice after either ovalbumin (OVA) sensitization and challenge or administration of αGalCer. iNKT cells from PD-L2$^{-/-}$ mice produced significantly more IL-4 than iNKT cells in vitro with mAbs resulted in significantly enhanced levels of IL-4 production. In contrast, PD-L1$^{-/-}$ mice showed significantly reduced AHR and enhanced production of IFNγ by iNKT cells. iNKT-deficient J18$^{-/-}$ mice reconstituted with iNKT cells from PD-L2$^{-/-}$ mice developd high levels of AHR, whereas mice reconstituted with iNKT cells form PD-L1$^{-/-}$ mice developed lower levels of AHR compared with control. As PD-L2 is not expressed on iNKT cells but rather is expressed on lung DCs, in which its expression is upregulated by allergen challenge or IL-4, these findings suggest an important role of PD-L2 on lung DCs in modulating asthma pathogenesis These studies also indicate that PD-L1 and PD-L2 have important but opposing roles in the regulation of AHR and iNKT-cell-mediated activation."[35].

Human iNKT cells and asthma. Bronchial asthma is associated with an inflammatory process that is characterized by the presence in the airways of large numbers of CD4$^+$ T cells producing IL-4 and IL-13. However, the CD4 antigen is expressed not only by class II MHC-restricted CD4$^+$ T cells, but also by a newly identified subgroup of T cells, CD1d-restricted natural killer T cells. These cells express a conserved (invariant) T-cell receptor and have a potent immunoregulatory function. Because mouse models of allergic asthma indicate that natural killer T cells are required for the development of allergen-induced AHR, and play an important role in human asthma. Akbari et al. used CD1d-tetramers, antibodies specific for natural killer T cells, as well as reverse-transcriptase-polymerase-chain-reaction analysis of the invariant T-cell receptor of natural killer T cells to assess the frequency and distribution of natural killer T cells in the lungs and in the circulating blood of 14 patients with asthma. About 60 percent of the pulmonary CD4$^+$CD3$^+$ cells in patients with moderate-to-severe persistent asthma were not class II MHC-restricted CD4$^+$ T cells but, rather, natural killer T cells. The natural killer T cells expressed an invariant T-cell receptor and produced type 2 helper cytokines. In contrast, the CD4$^+$ T cells found in the lungs of patients with sarcoidosis were conventional CD4$^+$CD3$^+$ T cells, not natural killer T cells"[6]. Thomas et al. "used endobronchial segmental allergen challenge in human atopic asthmatics to define the pattern of chemoattractant receptor expression on recruited T cells as well as the numbers of recruited CD1d-restricted NKT cells and levels of chemokines in the BAL fluid. CD1d-restricted NKT cells comprised only a small minority of BAL T cells before or after Ag challenge. BAL T cells were enriched in their expression of specific chemoattractant receptors compared with peripheral blood T cells prechallenge, including CCR5, CCR6, CXCR3, CXCR4, and BLT1. Following segmental allergen challenge, no chemoattractant receptor was specifically increased. However, CCR6 and CXCR3, which were expressed on virtually all CD4$^+$ BAL T cells prechallenge, were markedly decreased on all recruited BAL T cells following Ag challenge, suggesting that these receptors were internalized following encounter with ligand in the airway. They suggest a role for CCR6 and CXCR3, in conjunction with other chemoattractant receptors, in the recruitment of inflammatory T cells into the BAL during the allergic asthmatic response" [36]. Matangkasombut et al. reported that "patients with severe asthma had a significant increase in the number of BALF iNKT cells when compared with the number seen in nonasthmatic control subjects. Some, but not all, patients with well-controlled asthma had an increase in the number of BALF iNKT cells compared with the number seen in nonasthmatic control subjects. They conclude that iNKT cells are present in the BALF of some, but not all, patients with asthma. The specific number of iNKT cells present in the BALF was quite variable, but patients with severe asthma appeared to more consistently have an increase in BALF iNKT cells than patients with well-controlled asthma, the majority of whom did not have an increase in the number of BALF iNKT cells over that seen in nonasthmatic individuals"[37]. Koh et al. also investigated that "how blood iNKT cells are associated with atopy in asthmatic individuals. Peripheral blood mononuclear cells were isolated from 45 asthmatic subjects. iNKT cells were stained with 6B11 mAb, anti-TCRvα24 mAb, or αGalCer-loaded CD1d-tetramer and analyzed with flow-cytometric assays. Increased serum total IgE or one or more positive skin reactions to common allergens were used as atopic indexes. Asthmatic subjects with IgE >500 IU/mL showed lower frequency of CD4$^+$ 6B11$^+$ iNKT cells or CD4$^+$ Vα24$^+$

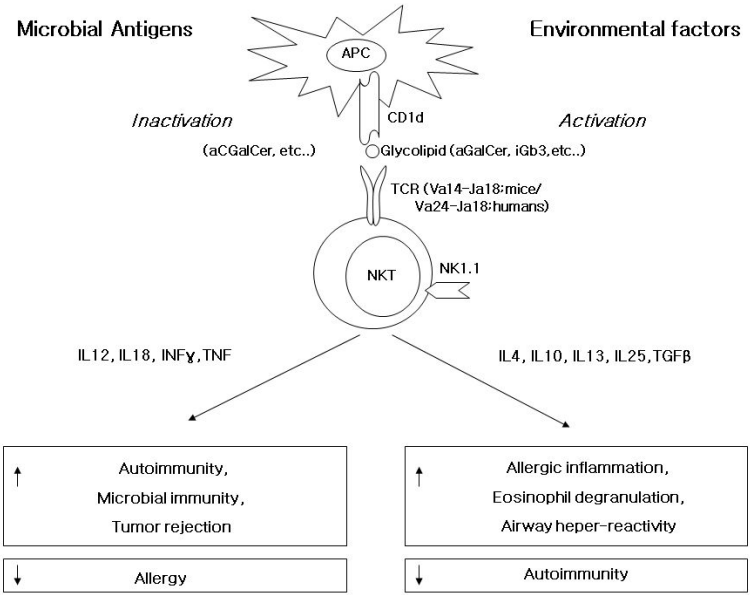

Fig. 2. Hypothetical model for the functional role of NKT cells. Exogenous or endogenous glycolipid-activate NKT cells produce IL-4 and IL-13, which induce airway hyperresponsiveness and in part enhance the function of CD4+ Th2 cells but, Th1- like NKT cells which produce Th1 but not Th2 cytokines, might protect against the development of asthma. Modified from Godfrey et al[19] and Umetsu et al[39].

iNKT cells compared with subjects with IgE<or=500 IU/mL. Asthmatic subjects with atopy on skin tests had lower frequency of $CD4^+$ αGalCer-loaded CD1d-tetramer$^+$ iNKT cells compared with those without atopy. The frequency of $CD4^+$ $V\alpha24^+$ iNKT cells was negatively correlated with total IgE in asthmatic subjects. Blood $CD4^+$ iNKT cells were inversely associated with atopic indexes in asthmatic individuals. They hypothesize that blood $CD4^+$ iNKT cells might behave like Th1-like iNKT cells in human asthma"[38].

Conclusion

NKT cell are expand the view of understanding about asthma and other allergic diseases that have variable mechanisms, not only peptide antigen but also, lipid antigen, microbial antigen, diesel, ozone, and other environmental causes. Multiple NKT subsets appear to exist, and multiple glycolipids appear to bind to the conserved, invariant TCR of iNKT cells, resulting in NKT cell activation. Thus, the role of iNKT cells may be extremely flexible, such that they can directly induce AHR or enhance Th2 sensitization to exogenous allergens. NKT cell are expected that can be used for the new target in the treatment of multiple forms of human asthma and allergic diseases through these investigates about subtypes of NKT cell and mechanisms of selective regulation (Fig. 2)[19, 39]. Many more experiments and studies are needed to clarify their roles in various forms of asthma.

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