Natural killer T-cells in asthma pathogenesis and treatment: old problems and future perspectives

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Shareable abstract (@ERSpublications)
NKT-cells are immunological cells expressing features of both innate and adaptive immunity, and might be involved in the pathogenesis of asthma. This article aims to reflect on past evidence and controversies. https://bit.ly/4iBPfQw

Cite this article as: Dilroba A, Fanella A, Pianigiani T, *et al*. Natural killer T-cells in asthma pathogenesis and treatment: old problems and future perspectives. *ERJ Open Res* 2025; 11: 01155-2024 [DOI: 10.1183/23120541.01155-2024].

Received: 11 Nov 2024
Accepted: 9 March 2025

Abstract

Natural killer T-cells (NKT-cells) are immunological cells expressing features of both innate and adaptive immunity, which have been reported to be involved in the pathogenesis of asthma and in the induction of airway hyperresponsiveness (AHR). In this review we discuss the controversial results obtained in the past due to the lack of standardised analytical methods and the inhomogeneity of cohorts. In recent years, the availability of more advanced techniques led to a significant improvement in the phenotyping of these cells. Several research studies, both in mice and humans, reported that NKT-cells might be involved in the induction of AHR through the secretion of cytokines. In mouse models, in the absence of NKT-cells, AHR was not triggered and the airway eosinophil count was reduced. A relationship between NKT-cells and both innate (*e.g.* dendritic cells) and adaptive cells (*e.g.* Tregs) was demonstrated as well. These cells are seemingly able to produce different sets of cytokines, depending on their micro-environment. Moreover, numbers of NKT-cells derived from bronchoalveolar lavage were higher compared to those from peripheral blood samples. Lastly, the possibility to administer novel monoclonal antibodies against several interleukin (IL) pathways (such as anti-IL-5 and anti-IL-13, which can both be secreted by NKT-cells) further places these cells at the core of the pathogenesis of asthma and highlights the need for further discussion.

Introduction

Asthma is a common respiratory disease that affects >300 million people worldwide with a prevalence of 10% in developed countries [1]. It is characterised by chronic and heterogeneous inflammation and a predisposition of the airways of patients to narrow excessively in response to specific or nonspecific inhaled stimuli, defined as airway hyperresponsiveness (AHR) [2]. From an immunological point of view, asthma can be classified according to the prevalent endotype of innate and adaptive immunological pathways and cell subsets expressed in the airways and associated to specific biomarkers or clinical features. Type 2 (T2) inflammation is regarded as the most prevalent phenotype in asthma and is characterised by T2-prevalent immune responses, including but not limited to eosinophilic proliferation and tissue infiltration, increased immunoglobulin (Ig)E production and an overproduction of T2-specific cytokines, such as interleukin (IL)-4, IL-5 and IL-13. On the other hand, non-T2 endotypes are typically associated with neutrophilic or paucigranulocytic inflammation and with an imbalance of adaptive immunology towards T helper (Th)1 and Th17 pattern [3]. According to this classification, the development of biologic therapies able to downregulate specific key cytokines has revolutionised the management of patients with severe asthma. However, the pathogenesis of asthma and the clinical and functional characteristics of related features, including AHR, are still to be fully clarified. Beyond eosinophils, neutrophils and lymphocytes, many other cell subsets, belonging both to the innate and adaptive immune system, have been reported to significantly contribute to the development and

perpetuation of different types of inflammation in asthma, to airway smooth muscle (ASM) hypertrophy/hyperplasia and to a dysregulated response to exposure to pathogens or pollutants. Among these, natural killer T-cells (NKT-cells) have recently been reported to be deeply involved in the development of asthma and in their connection with the induction of AHR [4, 5]. This review is focused on the role of NKT-cells in the development of asthma and the most relevant results obtained from animal models and human subjects in recent years. Past problems related to the technical difficulties in accurately detecting NKT-cells are also reported and discussed. Lastly, the possibility to administer novel monoclonal antibodies against several interleukin pathways (secreted also by NKT-cells) sheds light on this subset of cells and highlights the need for further discussion.

Old dilemmas and novel insights

The importance of the innate immune system was recently highlighted by several publications [6, 7]. The role of NKT-cells in the development of asthma has been investigated both in mice and in human subjects. Unfortunately, the findings of these studies appear overall controversial, leading to equivocal, unclear and sometimes contradictory results [8]. Most of the data available in the literature were published between 2004 and 2014, and in this decade two major limitations can be identified [8]. First, there is an intrinsic limitation to the methodology implemented for the detection of NKT-cells, which was mainly performed by flow cytometry [8]. In addition, the lack of a standardised flow cytometric gate strategy further limited these results [8]. In recent years, the possibility to use more advanced techniques, with the extended commercial availability of fluorochromes and the increased number of channels able to simultaneously detect a large number of parameters, led to a significant improvement in the phenotyping of these cells [9, 10].

Conveniently, a recent paper specifically focused on the methodological issues for the detection of NKT-cells, providing different protocols that classify these cells into distinct functional subsets based on the expression of transcription factors and the production of cytokines, both in animal and human models [11].

In the past, unspecific binding of the fluorochromes was also reported, which led to overestimation of NKT-cell numbers, with significant effects on the observed cell frequencies [11, 12]. The second problem relates to the classification of asthma, which in the last two decades has switched from a solely clinical perspective to a multidimensional approach, including immunological features.

Therefore, much of the evidence concerning expression and activity of NKT-cells in asthmatic patients may not take into account the evolution of pathogenic models of disease and appear to be “outdated”.

Considering these improvements, a better understanding of NKT-cell biology might be achieved.

Immunology of NKT lymphocytes

A large array of environmental factors, such as particulate matter, toxins, reactive oxygen species, chemicals, allergens and infectious microbes, are able to trigger the innate immune system leading to lung inflammation and asthma exacerbations [13]. This system comprises innate cells, including NKT-cells and innate lymphoid cells (ILCs), which produce a wide range of cytokines that contribute to T2 inflammation [14]. These cytokines are directly involved in asthmatic lung inflammation and therefore a target of novel monoclonal antibody therapies such as anti-IL-5 (benralizumab, reslizumab and mepolizumab) or anti-IL-4/13 (dupilumab) [15–17]. The specific roles of NKT-cells and ILCs in the pathogenic mechanisms leading to asthma are still to be defined, but their involvement has been frequently reported.

NKT-like cells represent <10% of circulating lymphocytes in adults and exhibit a high-density TCR-cluster of differentiation (CD)3 complex as surface marker, similar to classical T-cells, and a low density of the CD56 molecule, similar to cytotoxic NK cells [18]. About 95% of NKT-like cells are represented by invariant NKT-like cells (iNKT-cells).

Classifications of NKT-like cells

Classification of NKT-cells is based on TCR expression dividing NKT-cells into Type I and Type II.

Type I NKT-cells are called “iNKT” cells and express an invariant TCRα chain that recognises a glycosphingolipid called “α-galactosylceramide” (α-GalCer), presented by CD1d [19, 20]. Human iNKT-cells express a semi-invariant TCR, with the α chain consisting of an invariant Vα24-Jα18 chain paired with a Vβ11 chain, while in mice the TCR α chain consists of Vα14-Jα18 [19].

Type II NKT-cells use different TCRα and β chains that are reactive to a larger array of antigens, including glycolipids, phospholipids and hydrophobic antigens [21, 22].

TABLE 1 Effects of several cytokines on iNKT-like cells

Molecules and receptors	Effects on NKT-cells	References
IL-15 and IL-23	Cytokine release and proliferation of iNKT	[46]
Anti-CD137 and anti-CD2	Cytokine release and proliferation of iNKT	[46]
IL-12 and IL-18	Stimulate NKT-like cells to release IFN-γ	[46]
IL-23	Upregulation of CD56 expression on NKT-like cells	[46]
NKG2A/C-CD94 heterodimers	Recognise the nonclassical MHC-Ib molecule	[50]
TGF-β	Activation of Foxp3$^+$ iNKT-cells	[39]
Molecules and receptors	**Functions of NKT-cells**	**References**
IL-5 and IL-13	Release of large amount of these cytokines from iNKT-cells	[47]
IL-10	Released mainly from Treg-like iNKT-cells	[48, 38]
NKG2D receptor	NK cell cytotoxicity and co-stimulating TCR signalling in T-cells	[51, 52]
CD244$^+$ CXCR6$^+$	Release of IFN-γ and cytotoxic function	[30, 31]
IL-17, IL-21 and IL-22	Released mainly from Th17-like iNKT-cells	[36]
IL-21	Released mainly from Tfh-like iNKT-cells upon stimulation	[33]
Perforin and granzyme B	Released from iNKT-cells for cytotoxic activities	[46]

iNKT-like cell: invariant NKT-like cell; NKT-cell: natural killer T-cell; IL: interleukin; IFN-γ: interferon-γ; MHC: major histocompatibility complex; TGF-β: transforming growth factor β; Treg: T regulatory cell; NK: natural killer; Th: T-helper; Tfh-like: T follicular helper-like.

In peripheral blood iNKT-cells contribute to 0.01–0.2% of circulating T-cells [23].

iNKT-cells can be classified according to several more features, such as the cytokines and transcription factors released, similarly to T-cell classification: Th1-like iNKT-cell (iNKT1), Th2-like iNKT-cell (iNKT2), Th17-like iNKT-cell (iNKT17) and T follicular helper (Tfh)-like iNKT-cell [24]. Moreover, iNKT-cells can be further stratified based on the expression of CD4 and CD8 as double negative (DN) human iNKT-cells, CD4$^+$ iNKT-cell or double positive (CD4$^+$CD8$^+$) NKT-like cells [25–27].

From a phenotypic point of view, DN iNKT-cell subsets mainly express C-X-C chemokine receptor (CXCR)6, which plays a role in the homeostatic distribution of iNKT-cells in the lung [25, 28]. Retinoic acid-related orphan receptor γ (RORγ) remains a critical master regulator of iNKT-cells that guides not only the generation and differentiation but also the survival and homeostasis of this iNKT subset [29].

From a functional point of view, activated DN iNKT-cells exhibit an enhancement of interferon (IFN)-γ levels and of their cytotoxic function [30, 31]. CD4$^+$ iNKT-cells are instead associated with naïve T-cell immune responses and exclusively produce IL-4 and IL-13 upon stimulation [30]. Another key difference between CD4$^+$ iNKT-cells and DN iNKT-cells is that the former produces a higher quantity of intracellular IFN-γ, IL-4, IL-17 and tumour necrosis factor (TNF) [30].

In healthy individuals, Th1-like iNKT-cells are predominantly DN iNKT-cells [26, 32]. The activation of iNKT-cell subsets is modulated by several cytokines and receptors. The main mechanisms are reported in table 1.

Human Th17-like iNKT-cells secrete the proinflammatory cytokines IL-17, IL-21 and IL-22 when activated [33]. Tfh-like iNKT-cells, which preferentially secrete IL-21 upon activation, have also been described [33–35]. Tfh-like iNKT-cells interact with CD1d on the surface of dendritic cells thus upregulating several cytokines [36, 37].

T regulatory-like (Treg-like) iNKT-cells release the immunosuppressive cytokine IL-10 [38, 39].

Interestingly, it has been reported that iNKT-cells exhibit plasticity of their cytokine production, which means that they can change their phenotype during the course of their life [40].

IL-17-producing iNKT-cells were identified in the spleen, liver and lungs as NK1.1-negative iNKT-cells [29]. IL-17-producing iNKT-cells are mostly found in the CD4-negative compartment, so iNKT17 cells have been proposed to be phenotypically the same as CD4–NK1.1 cells [41].

While Type I NKT-cells' function has been well investigated, the functional role of Type II NKT-cells is less clear due to the lack of universal and specific staining antibodies [42]. In addition, different lipid antigens influence not only the magnitude but also the quality of NKT-cell activation [43].

A graphic representation of the classification and subtypes of NKT-cells is presented in figure 1.

Activation and effector function of NKT-cells

Activation of NKT-cells depends on the TCR binding, followed by proliferation of these cells and cytokines secretion, particularly IFN-γ and TNF-α. iNKT-cells are also able to activate antigen-presenting cells (APCs) early during immune responses. APCs then proceed to recruit an adaptive immune response during the infection process [44, 45]. van de Wetering [46] explains the role of several mechanisms involved in these processes.

In addition to the production of IFN-γ, activated NKT-like cells release higher amounts of TNF-α, IL-5 and IL-13 [47]. On the other hand, NKT-like cells barely produce any regulatory cytokines (IL-10) [48]. NKT-like cells are also strong producers of perforin and granzyme B, by which they exert cytotoxic properties [49].

NKT-like cells also express the NKG2A/C-CD94 heterodimers that recognise the nonclassical major histocompatibility complex (MHC)-Ib molecule [50]. NKG2D receptor activation enhances NK cell cytotoxicity while simultaneously co-stimulating TCR signalling in T-cells. It has been proposed that NKG2D triggering could be responsible for the majority of the MHC-unrestricted cytotoxicity of $CD8^+$ T-cells [51, 52].

NK and iNKT-like cells as drivers of asthma pathophysiology

The role of innate immunity and in particular the role of NK and iNKT-like cells has been described in asthma development and pathophysiology. Although several recently published novel research articles describe their role in this disorder, the molecular mechanisms involved need to be further analysed. Moreover, no up-to-date data for analysis of NK and iNKT-cells are reported.

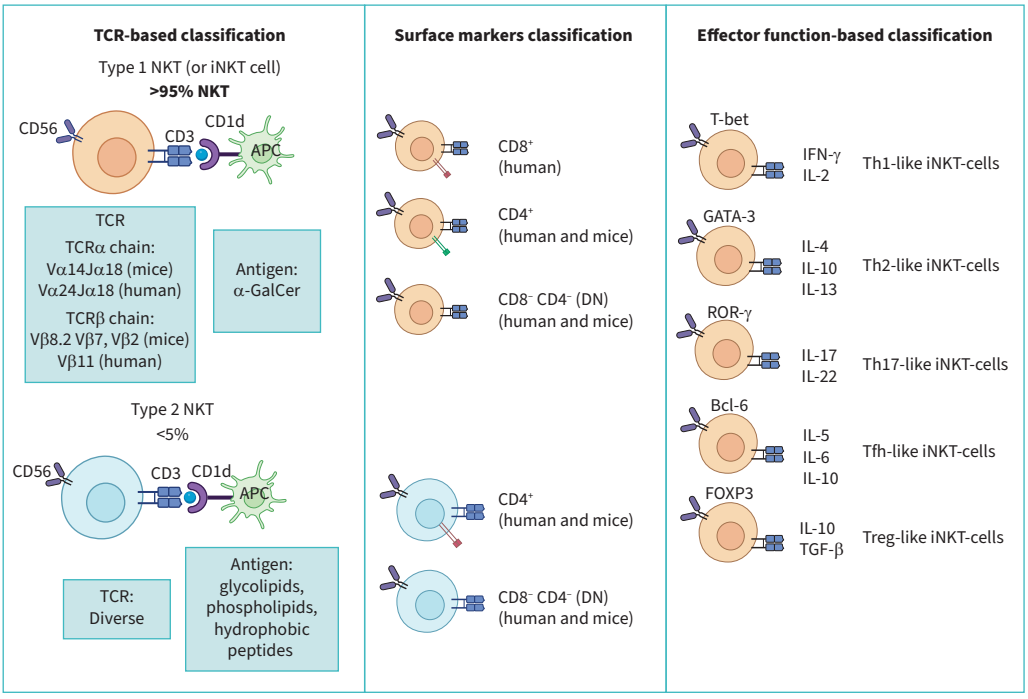

FIGURE 1 Comparison between the three main types of NKT-cell classifications: TCR-based classification, divided into Type 1 NKT-like cells, also known as iNKT, and Type 2; surface markers CD4 and CD8 classification; effector function classification, which involves only iNKT-like cells and is based on transcription factors. NKT-cell: natural killer T-cell; iNKT-cell: invariant NKT-like cells; Th: T-helper; Treg: T regulatory cell.

NK cells have been implicated in asthma pathogenesis, exhibiting both pro-inflammatory and regulatory roles that can either exacerbate or mitigate disease progression [53]. The current scenario posits a pleiotropic action of the NK cells, with regulatory and effector action that contributes to asthma's complex immune and inflammatory environment [54]. NK cells are important actors in the antimicrobial immune response, acting against bacterial and viral insults through the secretion of cytokines and chemokines [55]. However in asthma, NK cells exhibit impaired cytotoxic activity, a deficiency associated with dysregulated metabolic pathways, which may contribute to exacerbations after viral exposure. In asthmatic patients, a decrease in NK cell cytotoxicity can be further exacerbated by the administration of inhaled and systemic corticosteroids for the management of stable or acute disease, which disables NK cell functions [56].

Regarding NKT-like cells with particular focus on iNKT-like cells, different studies have shown contradictory results on the involvement of these cells in asthma. The role of iNKT-cells in asthma seems to correlate with both T2 high and T2 low forms of inflammation in asthma [57]. The latter is independent of adaptive immunity and associated with airway neutrophils [58]. Moreover, no available treatments exist for these kinds of patients. For these reasons, expanding knowledge regarding the role of these cells in asthma pathobiology is of primary importance. In the following paragraphs, the role of NKT-like cells is widely described.

iNKT-cells in mouse model of asthma

iNKT-cells and airway hyperreactivity in mouse model

Murine experimental models have served as an invaluable tool in revealing the connection between iNKT-cells and asthma pathology. The role of iNKT-cells in the pathogenesis of asthma was investigated by evaluating whether AHR develops in the absence of Vα14 iNKT-cells through the use of CD1d deficient mouse and Jα281$^{-/-}$ mice (which both lack iNKT-cells). It was found that in the absence of iNKT-cells, AHR was not triggered, and the airway eosinophil count was substantially reduced as well. Moreover, knockout mice showed fewer cells in their bronchoalveolar lavage fluid (BALF) with decreased eosinophil count, macrophages and neutrophils, while recruitment of lymphocytes appeared not to be modified [59, 60].

Interestingly, the absence of AHR was not determined by a deficiency in T2 responsiveness, or by an intrinsic inability of the mice to develop AHR, since when IL-13 (an IL that directly stimulates the contraction of ASM) was administered, a severe AHR response was observed in CD1d$^{-/-}$ mice as well [60]. The same study also demonstrated that after ovalbumin (OVA) challenge, iNKT-cells present an upregulation of CD69 when compared to OVA-immunised and saline-challenged mice [60]. Interestingly iNKT-cells seem to play a pathogenic role only regarding acute asthma: in mouse models of chronic asthma, following OVA challenge, both the wild-type (WT) mice and the CD1d$^{-/-}$ mice developed AHR and showed an increased cellularity in BALF, peribronchial and perivascular concentration and airway remodelling compared to the respective naïve counterparts [61].

In order to further prove the pathogenetic role of iNKT-cells in acute asthma, some authors sought to reconstitute knockout mice (Jα281$^{-/-}$ which lacks iNKT-cells) by transferring WT-NKT-cells before OVA airway challenge, which restored the capacity of the Jα18 mice to develop AHR as well as the level of Th2 cytokines in the BALF and the production of serum IgE [59, 60].

iNKT-cells and interaction with other immunological cells

iNKT-cell deficiency is linked with a decreased expression of surface maturation markers and proinflammatory cytokine secretion by lung dendritic cells (LDCs) in mice immunised and challenged with OVA. On the contrary, stimulation with α-GalCer, a glycolipid that specifically binds and stimulates iNKT-cells' activation, causes an increase in the expression of apoptotic receptors when compared to controls [62]. These results were also confirmed by transferring iNKT-cells from WT mice immunised and challenged with OVA or house dust mite into CD1d$^{-/-}$ mice, which resulted in an increased expression of surface markers and concentration of proinflammatory cytokines [62].

Moreover, this study also demonstrated that immature dendritic cells underwent maturation upon interaction with iNKT-cells in the presence of OVA, but in its absence, they adopted a tolerogenic phenotype. The mechanism underlying this interaction was assessed by measuring the level of CD40-l, the major ligand that iNKT-cells express when activated. LDCs isolated from WT mice immunised and challenged with OVA are then capable, *in vitro* experiments, of priming Th2 responses, contrary to CD1d$^{-/-}$ mice, which also showed lower levels of IL-4, IL-5 and IL-13 [62].

Akbari *et al.* [60] also found that iNKT-cells have a primary role in guiding the development and maturation of Th2 cells: transferring OVA-sensitised Th2 cells from Jα281 ko mice into severe combined

immunodeficient mice (SCID mice) does not restore AHR. This result demonstrated that Th2 cells can also develop and activate in the absence of iNKT-cells.

NAGATA *et al.* [63] found that iNKT-cells express the thymic stromal lymphopoietin (TSLP) receptor and that once activated by its ligand, iNKT-cells start to proliferate and produce IL-13.

iNKT-cells have also been reported to be significantly influenced by the regulatory compartment of the immune system. Treg cells are suppressor cells that once activated modulate the immune system and prevent allergic diseases [64]. Upregulation of CD39 increases iNKT-cells in the spleen and the peripheral blood, and decreases iNKT-cells in the lung, while administration of anti-CD25 reverses the effect. This notion suggests that Treg cells can regulate the migration of iNKT-cells through CD39 in an OVA-induced asthma mouse model. Consistent with the ratio of iNKT-cells in lung, upregulation of CD39 causes a decrease in the levels of IL-4 and IFN-γ. A study by CHUANG *et al.* [65] demonstrated that DN iNKT-cells express many cytotoxicity-related molecules, including perforin and granzyme, and might interact with CD4+ T-cells through mechanisms sustained by programmed cell death protein 1 ligand (PD-L1) mechanisms, because anti–PD-L1 monoclonal antibodies partially block the *in vitro* activity of the iNKT-cells [66–68].

A summarised representation of the types of interactions carried out by NKT-cells is given in figure 2.

It is interesting to note that the varying studies carried on different asthma models (ozone-induced, viral-induced, air pollution-induced and allergen-induced) singularly involve different subsets of

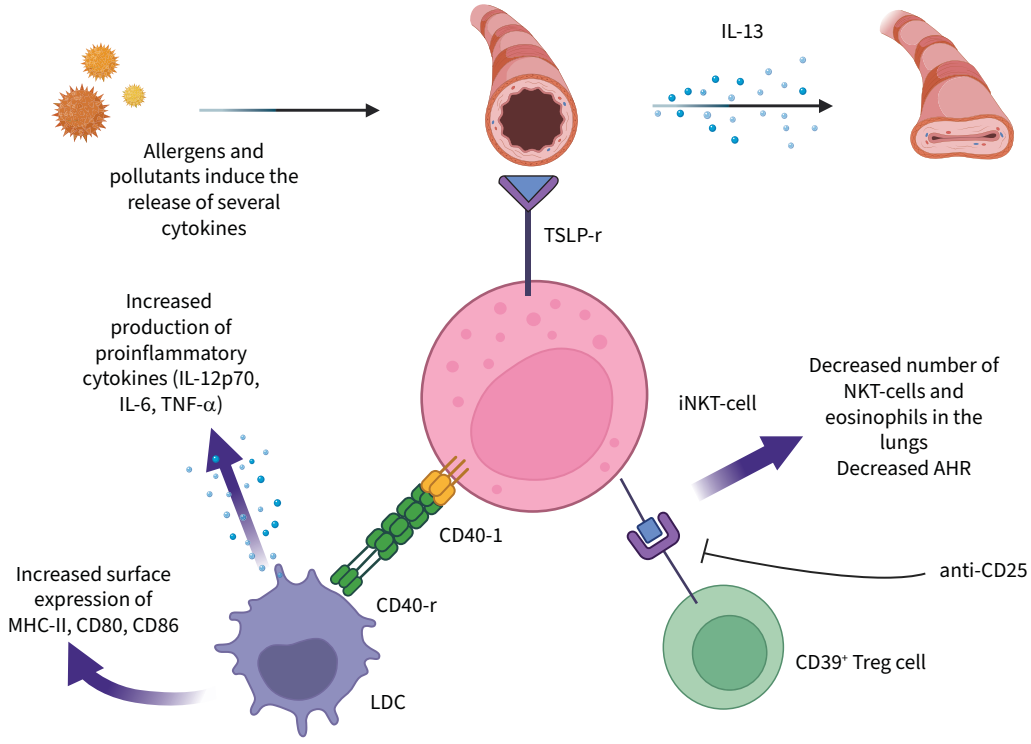

FIGURE 2 Interactions between NKT-cells and other immunological cells. This picture summarises the types of interaction carried out by iNKT-cells and other immunological cells. Lower left side: iNKT-cells express the CD40 ligand (CD40L) which binds to the CD40 receptor (CD40R) expressed on lung dendritic cells (LDC); this interaction causes the increased expression of MHC-II, CD80 and CD86, which represent surface maturation markers of LDC cells. The interaction also induces the release of proinflammatory cytokines. Top: allergens and pollutants in asthma models induce the release of several types of cytokines and interleukins (IL). iNKT-cells express the TSLP receptor, which when activated by its ligand induces the production of IL-13, which can directly induce airway hyperreactivity (AHR) and bronchoconstriction. Lower right side: Treg cells can regulate the migration of iNKT-cells through CD39 thus reducing their concentration in the lungs, whereas antiCD25 reverses the effect. iNKT-cell: invariant natural killer; NKT-cell: natural killer T-cell; Treg: T regulatory cell; TSLP: thymic stromal lymphopoietin; TNF-α: tumour necrosis factor α; MHC: major histocompatibility complex.

iNKT-cells, both with a preventive or augmenting role, further underscoring the complexity and heterogeneity of immunological dysregulation in asthma [65, 69–71].

iNKT-cells in asthmatic patients

Percentage of iNKT-cells in BALF and peripheral blood from asthmatic patients

Mouse models of asthma cannot replicate all features of human asthma, and the only available method to evaluate the role of iNKT-cells in humans is the direct assessment of iNKT-cell activity in the peripheral blood and in the lungs of patients with asthma.

Akbari *et al*. [72] used CD1d tetramers loaded with α-GalCer to assess the frequency and distribution of iNKT-cells in the lungs, BALF and blood of 14 patients with moderate to severe asthma. By using tetramer staining they found that in asthmatic patients, 63% of the $CD4^+$ cells in BALF were iNKT-cells expressing the invariant TCR Vα24, and similar results were obtained from biopsy samples. In sarcoidosis patients and in healthy controls, <1% of the $CD4^+$ cells expressed the invariant TCR Vα24. It was also found that the iNKT-cells in the lungs of asthmatic patients produced both IL-4 and IL-13, but not IFN-γ. In peripheral blood, the iNKT-cells constituted <0.1% of the mononuclear cells and <1% of the $CD4^+$ cells. The observation that >90% of the iNKT-cells resident in the lungs are $CD4^+$ cells and only 50% of the iNKT-cells in peripheral blood are $CD4^+$ cells suggests that a subgroup of iNKT-cells is recruited and enriched in the lung [72]. On the contrary, studies on the airways detected a smaller number of iNKT-cells, and those on BALF (using the Vα24 and Vβ11 monoclonal antibodies and CD1d tetramers loaded with α-GalCer) showed that iNKT-cells were <2% of the $CD3^+$ cells and <1.5% of the $CD3^+CD4^+$ cells. Moreover, the occurrence in BALF samples of macrophages, monocytes, and dead or dying cells which nonspecifically pick the staining of monoclonal antibodies is a source of false positive results in flow cytometry. To exclude these cells, Vijayanand *et al*. [73] analysed their morphological features and used a DNA-binding reagent for their staining.

Owing to persistent activation by a self-ligand, keeping the iNKT-cells poised to release cytokines or kill target cells at any time, the iNKT-cells are the only known immune cells that express the early activation marker CD69 continuously. However, it could be hypothesised that iNKT-cells become activated during a short period of time and rapidly increase in number, then they could disappear from detection because of downregulation of their TCR and progress within a few days to activation-induced cell death [74].

iNKT-like cells activation in asthmatic patients

iNKT-cells are activated by endogenous glycolipids that become expressed in the inflammatory environment that is elicited by the protein antigen and Th2 cells. Exogenous glycolipids from microorganisms or plant pollen that enter the lungs might activate iNKT-cells directly and cause wheezing [75].

iNKT-cells have two pathways of activation: through CD1d and through CCR9 and CCL25, with consequential production of IL-4 and IL-13. $V\alpha24^+$ iNKT-cells in the blood of asthmatic patients selectively express CCR9; accordingly, a great number of $CCR9^+$ and $V\alpha24^+$ cells are present in bronchial biopsy samples. In asthma, conventional $CD3^+$ αβ T-cells could be polarised to a Th2 phenotype by cell-to-cell contact with $V\alpha24^+$ iNKT-cells, with enhanced expression of CCR9. This induction requires CCL25 and CCR9 to activate adjacent membrane signalling by CD226, a leukocyte-adhesion molecule that is widely expressed on mononuclear cells. The CD226 appears to be critical in activating $V\alpha24^+$ iNKT-cells for the induction of this Th2 bias in $CD3^+$ T-cells [76]. A graphical representation of the activation pathways of the NKT-cells is shown in figure 3.

Ikegami *et al*. [77] had already found that the number of circulating iNKT-cells and their frequency among $CD3^+$ T-cells was decreased in patients with asthma in comparison with healthy controls. iNKT-cells from patients with asthma had no intrinsic proliferative defect after stimulation with α-GalCer, and the number of iNKT-cells was not influenced by atopic status or severity of disease. In addition, no correlation between the number of peripheral NKT-cells and clinical variables such as number of eosinophils, serum IgE levels, forced expiratory volume in 1 s (FEV_1) %, age and duration of asthma were found. However, it is possible that the decrease in iNKT-cells could facilitate the development of allergic diseases.

In contrast with these results, Koh and colleagues [78] showed that the number of circulating iNKT-cells was reduced in atopic asthmatic individuals and was negatively related with serum total IgE levels in asthmatic subjects, indicating that blood iNKT-cells may be inversely associated with atopy in human asthma.

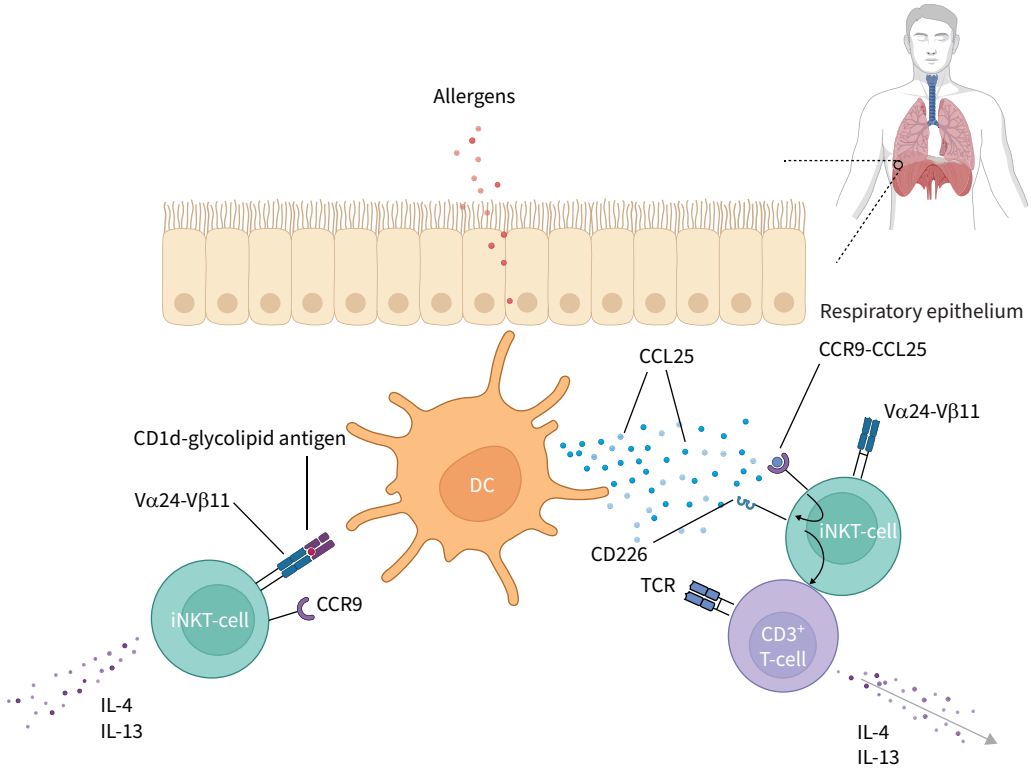

FIGURE 3 Two pathways of activation of iNKT-cells: through CD1d (on the left) and through CCR9 and CCL25 (on the right), with consequential production of IL-4 and IL-13. Conventional $CD3^+$ αβ T-cells can be polarised to a Th2 phenotype by cell-to-cell contact with $Vα24^+$ iNKT-cells, with subsequent enhanced expression of CCR9. This induction requires CCL25 and CCR9 to activate adjacent membrane signalling by CD226. iNKT-cell: invariant natural killer T-cell; DC: dendritic cell.

The results of a study examining BALF samples from asthmatic patients with a broad range of severity and symptom control suggested that these parameters might be loosely related to the number of pulmonary iNKT-cells. However, a higher number of pulmonary iNKT-cells was observed in patients with severe asthma and only in some patients with well-controlled asthma, compared to non-asthmatic controls. These results suggest that the absolute number of iNKT-cells present in the lungs may not be as important as their functional capacity – once activated – to contribute to the development of AHR [79].

iNKT-cells during asthma exacerbations

Koh and colleagues [80] conducted a real-life study to evaluate iNKT-cells behaviour in peripheral blood during asthma exacerbation. The results showed a decreased number of iNKT-cells during infection-triggered asthma exacerbation – which was not observed after a positive allergen challenge [81]. iNKT-cells inversely correlated with sputum eosinophils and Th1 and Th2 cytokines, suggesting that they may play an important role in the immune pathogenesis of acute asthma exacerbations in humans [80].

iNKT-cells might be recruited from the bloodstream to the airways and lung tissue by chemokines produced during asthma exacerbation. An increase of macrophage inflammatory protein (MIP)-1β (a ligand of CCR5), which is expressed on blood NKT-cells in humans, was reported in sputum supernatant [80]. The reduction of blood iNKT-cells during asthma exacerbation and their association with the development of eosinophilic airway inflammation was reported by Shim *et al.* [82], who showed that blood iNKT-cells produced more IL-4 in asthmatic patients compared to normal controls and that the number of IL-4 secreting iNKT-cells in peripheral blood was inversely related to lung function. This result suggested that iNKT-cells belong to the T2-inflammation subset.

On the other hand, Koh *et al*. [78] showed that the ratio of $CD4^+/CD3^+$ iNKT-cells was inversely related to the atopic indexes (increased serum total IgE or one or more positive skin reactions), which indicates that blood $CD4^+$ iNKT-cell subsets are likely to promote Th1 responses in human asthma.

Blood $CD4^+$ iNKT-cell subsets might differ functionally from lung $CD4^+$ iNKT-cells. Thus, these cells might function differently according to cellular location. We can speculate that human blood $CD4^+$ iNKT-cells might behave as Th2-like iNKT-cells before the development of asthma and allergic diseases but as Th1-like iNKT-cells after establishment of the diseases [78].

Conclusions and future perspectives

Multiple inflammatory and clinical phenotypes of asthma have been recently discovered and investigated. A central feature common to all forms of asthma is AHR [83]. Many inflammatory and immunological pathways related to the pathogenesis of asthma have been shown to require the presence of CD1d-restricted, invariant T-cell receptor-positive iNKT-cells. Recently introduced monoclonal antibodies target cytokines involved in the pathogenesis of asthma which are also released from iNKT-cells. For all these reasons it is of cardinal importance to improve the knowledge regarding the immunology of NKT-cells, their recruitment and possible interaction with the novel target therapies in asthma patients.

Provenance: Submitted article, peer reviewed.

Conflict of interest: No competing interest to declare.

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