Role of type 1 natural killer T cells in pulmonary immunity

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Mucosal sites are populated by a multitude of innate lymphoid cells and “innate-like” T lymphocytes expressing semiconserved T-cell receptors. Among the latter group, interest in type I natural killer T (NKT) cells has gained considerable momentum over the last decade. Exposure to NKT cell antigens is likely to occur continuously at mucosal sites. For this reason, and as they rapidly respond to stress-induced environmental cytokines, NKT cells are important contributors to immune and inflammatory responses. Here, we review the dual role of mucosal NKT cells during immune responses and pathologies with a particular focus on the lungs. Their role during pulmonary acute and chronic inflammation and respiratory infections is outlined. Whether NKT cells might provide a future attractive therapeutic target for treating human respiratory diseases is discussed.

INTRODUCTION

Mucosae are continuously exposed to self and environmental triggers and serve as a first line of defense against various pathogens and insults. Establishment of immune responses at these sites needs to be tightly regulated in order to contain and/or eliminate pathogens, to generate effective memory against them, and to prevent the development of uncontrolled inflammation and/or autoimmune diseases. Considering this, the nature and distribution of immune cells in mucosal tissues are of utmost importance. Among “innate-like” T lymphocytes that populate these sites are mucosal-associated invariant T cells, \( \gamma \delta \) T lymphocytes and natural killer T (NKT) cells expressing a semiconserved T-cell receptor (TCR).

NKT cells are a unique subset of thymus-derived T cells that carry a functional antigen (Ag) receptor but also display similarities with innate immune cells through their rapid activation in response to multiple lipid Ags and/or cytokines. Despite their relative low numbers at mucosal sites, NKT cells act as sentinels during infections to elicit early host defense mechanisms and sometimes can be involved in the pathogenesis in a variety of diseases. In this review, we will focus on current understanding and gaps in knowledge regarding NKT cell functions in pulmonary immunity.

Definition, development, and homeostasis

NKT cells represent a population of innate/memory unconventional T lymphocytes that co-express markers typically found on NK cells and conventional T lymphocytes. Unlike conventional T lymphocytes, which recognize peptide Ags, NKT cells recognize a broad range of endogenous and exogenous lipid Ags presented by the monomorphic major histocompatibility complex class I-like CD1d molecule. CD1d-restricted NKT cells are divided into two major subsets (Table 1) based on their TCR repertoire and antigenicity profile.\textsuperscript{1–3} By far, the best-characterized NKT cell subset is the “type 1 NKT cell” population. They express an invariant TCR-\( \alpha \) chain (V\( \alpha \)14-J\( \alpha \)18 in mice and V\( \alpha \)24-J\( \alpha \)18 in humans) paired with a limited number of \( \beta \)-chains (V\( \beta \)8.2, V\( \beta \)7, or V\( \beta \)2 in mice and V\( \beta \)11 in humans). An important feature of type 1 NKT cells is that, after endogenous or exogenous TCR-mediated activation, they promptly produce a wide array of cytokines and chemokines.\textsuperscript{4} Through this property, type 1 NKT cells are viewed as important cells to conduct early effector and regulatory functions.

The second major subset of NKT cells is termed type 2 NKT cells. These cells have a more diverse TCR repertoire and recognize an almost completely different pattern of CD1d-associated ligands compared with type 1 NKT cells.\textsuperscript{5,6} Although

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Abbreviations: Ags, antigens; α-GalCer, α-galactosylceramide; NKT, natural killer T cell; TCR, T-cell receptor.

| Table 1 | Major populations of CD1d-restricted NKT cells |
|---------|---------------------------------------------|
| NKT cell family | Type 1 NKT cells | Type 2 NKT cells |
| TCR-α chain | Vα14, Jα18 (M) | Vα3, 2, Jα8 (M) and some |
| | Vα24, Jα18 (H) | γδ T cells |
| TCR-β chain | VB7.2, VB8.2 (M) VB11 (H) | Diverse including VB8.2 |
| Markers defining subsets | CD4, NK1.1 (M) CD4, CD8 (H) | CD4 |
| α-GalCer reactivity | Yes | Yes |
| Other Ags | β2G3, α-GlcCer, β-GlcCer, β-GalCer, α-GalDAG, α-GlcDAG, β-GalCer | Sulphatide, Cardiolipin, α-GlcDAG, β-ManCer |

recent progresses have been made concerning the functions and characterization of these cells, the lack of specific molecular markers preclude any thorough investigation at the moment. Nevertheless, this population has been associated with strong immunomodulatory properties in many settings including autoimmunity, infections, and cancer.5,7 More recently, an atypical population of NKT cells (termed type 1a NKT cells) that does not fit perfectly in the type 1/type 2 classification has been described.8 These cells express a semi-invariant Vα10/11.50 TCR and, similarly to type 1 NKT cells, they recognize α-galactosylceramide (α-GalCer) in a CD1d-dependent manner. However, the functions of this subset remain to be determined. In this review, we will focus our interest on type 1 NKT cells (referred here as NKT cells).

NKT cells originate from the thymus where they are selected by endogenous lipids presented by the CD1d molecules expressed by double-positive thymocytes.9 After several rounds of cell division, NKT cells acquire a memory/effector phenotype before thymic emigration. Recent data demonstrated that NKT cells require post-thymic education by commensal microbiota to fully mature and to acquire their functionality.10,11 NKT cells are found in various peripheral organs including the liver (~30% of T cells in mice, ~1% in human), spleen (10-fold less), lymph nodes (~0.1–0.5% of lymphocytes), and bloodstream (~0.2% of circulating cells). They are far more abundant in mice than in humans, a likely consequence of the lack/low frequency of CD1a/b/c-restricted T cells and mucosal-associated invariant T cells in mice. For still undetermined reasons, there is a high frequency of NKT cells in the omentum in humans (~10% of T cells).12 Mucosae are also populated with NKT cells as they have been described in lungs, intestine, and reproductive tracts. At steady state, mouse NKT cells represent ~5–10% of the T-cell pool in the lungs where they constitutively reside in the microvasculature with a broad distribution in peribronchial and interalveolar capillaries.13 Information from human studies is less compelling, although NKT cells have been detected in BAL14 and sputum15 at very low frequencies (0.1–0.2% of T cells).

Functions and classification
NKT cells recognize exogenous and endogenous (glyco)lipids presented by the CD1d molecule expressed by Ag-presentation cells, including dendritic cells (DCs). The prototypic NKT cell activator in mice and humans is α-GalCer, an α-linked glycosphingolipid originally isolated from a marine sponge.16 In parallel, NKT cells can be activated by microbial lipids and, to a lesser extent, by stress-induced self-lipids. In response to TCR engagement, NKT cells promptly release a wide array of cytokines including interferon (IFN)-γ, interleukin (IL)-4, and/or IL-17 and upregulate several co-stimulatory molecules. These events contribute to the reciprocal maturation of surrounding Ag-presenting cells, for instance, the release of activating cytokines (e.g., IL-12 and IL-18), and ending up with the downstream activation of NK cells, neutrophils, macrophages, γδ T cells and B and T lymphocytes with important outcomes on the immune response.17–22 NKT cells also exert cytolytic activity through granzyme B secretion and FAS ligand-induced apoptosis mechanism.23 According to the nature of cytokines produced, NKT cells differently influence the character and the strength of innate and acquired immune responses. Of importance, NKT cells also have a part in tolerance induction, thus highlighting their functional plasticity.24–27 Through these unique properties, NKT cells influence host defense responses and pathologies including autoimmune diseases, inflammation, resistance to tumors, and antimicrobial responses with either protective (tumor resistance, infections) or deleterious (inflammatory diseases) effects.1,3,28

NKT cells promote/orientate or suppress immune responses, presumably owing to the existence of distinct subsets with specialized functions.1,3,9 Several distinct populations of NKT cells, based on CD4, NK1.1, and IL-25 receptor (IL-17RB) expression have been reported and differ in their cytokine profile and functions (Figure 1). The CD4+ subset is associated with a more pronounced ability in producing T helper 1(Th1)-related cytokines and is proposed to bear a marked antitumor activity relative to their CD4+ counterparts.29,30 Although CD4+ NKT cells produce both Th1- and T helper 2 (Th2)-related cytokines,30 expression of IL-17RB defines cells with a higher capacity to secrete IL-13 and IL-4 but little IFN-γ, an important property in Th2-mediated diseases.31,32 Finally, the NK1.1−CD4+ NKT subset expressing the retinoic acid receptor-related orphan receptor-γt produces high amounts of IL-17A33,34 and IL-22.35–37 Unlike the mouse system, human develops a CD8+ NKT subset, although its functional impact has to be defined.

NKT cell subtypes have a preferential distribution pattern that fits nicely with their putative functions. For example, NK1.1− retinoic acid receptor-related orphan receptor-γt+ NKT cells (a subset that co-expresses CCR6) are enriched in skin and peripheral lymph nodes and to a lesser extent in the lungs.34,35 Regarding the critical role for IL-17 in mucosal immunity,30 this pattern of distribution is not surprising. NKT cell diversity and localization can partially explain the pleiotropic, but not redundant, functions of these cells,
although other factors are likely to be required. Among them, the nature of Ags recognized by NKT cells as well as the microenvironment in which NKT cells primarily become activated are critical.

**Role of NKT cells in humans**

NKT cells are less frequent in humans compared with the mouse system, and their proportion in blood, and possibly within organs, greatly vary according to the donor. Although it is well admitted that NKT cells mediate both beneficial and detrimental outcomes in the mouse system, it is also recognized that intrinsic NKT cell defect (deficiency or impaired cytokine production) is associated with human diseases such as multiple sclerosis, graft-vs.-host disease, rheumatoid arthritis, systemic lupus and type 1 diabetes. A defect in NKT cells has also been widely described in solid tumors and hematological malignancies and might predispose to infectious diseases such as acute tuberculosis and Epstein–Barr virus. On the other hand, there are situations where NKT cells seem to be pathogenic such as atherosclerosis, allergy, and skin disorders. Clinical trials have been conducted to harness NKT cell functions for therapeutic purposes, particularly in cancer patients. Although α-GalCer administration leads to a significant immunological response and is rather well tolerated, the clinical benefits are still limited. Therefore, future progresses are clearly required to increase the efficacy of NKT cell-based therapies. Aside of this, there is an increasing interest in order to manipulate NKT cells to improve vaccine efficacy and to treat autoimmune and inflammatory diseases.

**Mechanisms of NKT cell activation**

During stressful conditions, including cell transformation, injury, inflammation, and infection, NKT cells rapidly become activated to exert their effector/regulatory functions. The mode of NKT cell activation has been principally studied during infection. NKT cells can respond to diverse microbial pathogens ranging from viruses to helminthic parasites. The pattern of Ags recognized by NKT cells is indeed larger than initially believed, as the TCR is likely to act more as a pattern recognition receptor. Indeed, NKT cells can be activated by various naturally occurring microbial (mostly

| Subsets in periphery | Cytokine profile/Features |
|----------------------|---------------------------|
| **Type I NKT family** |                          |
| Va14Ja18 TCR (M)     |                           |
| Va24α18 TCR (H)      |                           |
| CD1d restricted α-GalCer recognition |         |
| CD4⁺ NKT             | RORγT⁻ | - Tₐ,17- and Tₐ,22-related cytokine production. |
|                      | NK1.1⁺ NKT | - Involved in mucosal immunity against pathogens. |
|                      | IL-17R⁺ | - Tₐ,1-related cytokine production. |
|                      | IL-17R⁺ CD4⁺ NKT | - Potent anti-tumor activity. |
|                      | IL-17R⁺ CD4⁺ NKT | - Tₐ,2-related cytokine production but little Tₐ,1. |
|                      | IL-17R⁺ CD4⁺ NKT | - Involved in inflammatory Tₐ,2-mediated diseases (AHRI induction). |
|                      | IL-17R⁺ CD4⁺ NKT | - Both Tₐ,1- and Tₐ,2-related cytokine production. |
|                      | CD8⁺ NKT | - Only found in Humans (under homeostatic conditions). |
|                      |          | - Cytokine profile and existence of subsets with specialized functions are still elusive. |

**Figure 1** Subsets of peripheral type 1 natural killer T (NKT) cells and associated functions.
bacterial) lipids presented by CD1d. For instance, the Gram-negative, lipopolysaccharide-lacking, bacteria *Sphingomonas*, express NKT cell Ags including α-glucuronosylceramide and α-galacturonosylceramide. In addition, other glycolipids and glycerophospholipids from *Borrelia burgdorferi*, *Mycobacterium bovis* Bacillus Calmette–Guerin, and *Streptococcus pneumoniae* can also directly activate NKT cells with or without any other factors. NKT cells also have the ability to respond to innate or inflammatory stimuli in conjunction, or not, with self or microbial Ag. In fact, the innate-cytokine driven activation seems to be the dominant pathway for NKT cell activation in response to virtually all infectious agents, especially viruses that do not produce lipids. Innate sensors, including toll-like receptors (TLRs), have a crucial role in the activation of sentinel cells and in the subsequent stimulation of NKT cells. For instance, triggering of TLR4, TLR7, or TLR9 in DCs leads to NKT cell activation. In this setting, IL-12, and other cytokines such as IL-18 or IFN type 1 stimulate NKT cells to produce IFN-γ, in concert or not with self-lipids. Other innate sensors involved in the recognition of microbe-conserved components, including retinoic acid-inducible gene-like helicases and NOD-like receptors might also contribute in the indirect activation of NKT cells, although this has been poorly investigated so far. The precise identity of self-lipids recognized by NKT cells remains unclear, but β-linked glycosphingolipids are good candidates. Among them, β-β-glucopyranosylceramide, isoglobotrihexosylceramide, and diisialoganglioside might serve as self Ags in some circumstances. In parallel, self-phospholipid Ags such as (lyso)phosphatidyethanolamine, phosphatidylinositol, and phosphatidylcholine might have physiological functions.

Various types of soluble stimuli can activate NKT cells to produce IFN-γ in the body but far less studies show that NKT cells produce IL-4 or IL-17 during infection. This situation is likely to occur *in vivo*, depending on the nature of the threat and the organ of interest, yet the mechanisms by which they do so are poorly defined. Similarly, the regulatory role of NKT cells in tolerance and immune suppression presumably requires some forms of activation, which remains to be determined.

NKT CELLS IN INTESTINAL AND URINARY/REPRODUCTIVE TRACT IMMUNITY

NKT cells populate the digestive tract and have been detected in the lamina propria (~1% of total lymphocytes) and to a lesser extent, in the intraepithelial lymphocyte compartment of both small and large intestine. Of note, numerous immune cells in the intestinal tissue display NKT cell phenotypic hallmarks that render mandatory the use of CD1d/α-GalCer tetramers for a meaningful analysis. Consistent with their regulatory functions, intestinal NKT cells have been evoked in the participation of oral tolerance by inducing tolerogenic DCs and by positively influencing the development of regulatory T cells. On the other hand, mouse NKT cells seem to be implicated in peanut allergen-induced eosinophilic esophagitis. Moreover, the presence of Th2-biased NKT cells and the high level of CD1d expression in the duodenum of patients with cow’s milk hypersensitivity suggested a potential participation of NKT cells in the pathogenesis of food allergy in humans. NKT cells also have a significant role in the development of gut inflammation. Their beneficial vs. pathogenic role in experimental inflammatory bowel diseases is dependent on the model used. In this context, it appears that NKT cells are generally protective during Th1-mediated pathologies (Crohn disease-like models) and harmful during Th2-mediated diseases (ulcerative colitis models). Surprisingly, relatively few studies have analyzed the role of NKT cells during oral infections. Despite clear signs of activation in response to various intestinal pathogens including the bacterium *Listeria* and the protozoan parasite *Toxoplasma*, their natural contribution to the control of intestinal infections is not obvious and controversial.

Less information is available about the role of NKT cells in immunity of the reproductive tract. However, early IFN-γ production by NKT cells contributes to the innate protection against genital herpes simplex virus type 2 infection leading to an enhanced survival and a reduced viral burden. NKT cells are also locally activated following genital tract *Chlamydia* infection and participate to the bacterial clearance. However, by eliciting this inflammatory response, NKT cells have a dual role in the pathology by influencing the development of fibrosis. Finally, exogenous activation of NKT cells by means of α-GalCer induces a protective role against genital tract *Chlamydia muridarum* infection.

NKT CELLS IN PULMONARY INFLAMMATION AND LUNG DYSFUNCTION

NKT cells have been shown to be required for pulmonary disorder in many experimental systems. The mechanisms involved vary according to the model used and the nature of the initial trigger. Here, we will review the role of NKT cells in acute lung injury and fibrosis and on asthma and chronic obstructive pulmonary disease (COPD; Table 2), two major chronic pathologies responsible for high levels of mortality and/or morbidity worldwide and for which there is an increase need for the development of new therapies.

Hypersensitivity reactions and asthma

Asthma is a common disabling inflammatory respiratory disease that affects millions of people worldwide. This is a complex disease with several distinct forms that might be associated with several different pathogenic mechanisms. Allergic asthma is the most common form of the disease. Its development occurs against environmental allergens and is characterized by a Th2-polarized immune response, eosinophil infiltration, high levels of specific IgE, mucus hypersecretion, and airway hyperreactivity (AHR), a cardinal feature of asthma. Non-allergic asthma has common pathological features with allergic asthma, including AHR. Recent advances led to the discovery that environmental factors, including viruses and air pollution, and Th2-independent components, such as neutrophils, IFN-γ, and IL-17, contribute to the pathogenesis of...
| Pathology               | Subset involved | Cytokine secretion | Mechanisms involved in NKT cell activation | Functions of NKT cells | Phenotype in NKT cell-deficient mice | α-GalCer (route of administration) | References |
|------------------------|-----------------|--------------------|-------------------------------------------|------------------------|-------------------------------------|------------------------------------|------------|
| **AHR**                |                 |                    |                                           |                        |                                     |                                    |            |
| Virus-induced (Sendai virus) | CD4⁻ NKT       | IL-13              | CD1d-dependent                             | Activation of IL-13-producing macrophages | Decrease in mucous cell metaplasia and AHR | Not tested | 97         |
| Ozone exposure         | Undetermined    | IL-17A             | CD1d-dependent                             | Lung injury and AHR development | No AHR development                   | Not tested | 96         |
| α-GalCer-induced       | Various         | IL-4/IL-5/IL-13    | CD1d-dependent                             | Promotion of eosinophilia and neutrophilia | NA                                  | NA                    | 13,34,92–95 |
| IL-25-induced          | CD4⁺ IL-17RB⁺ NKT | IL-4/IL-13         | Direct activity of IL-25 on IL-17RB⁺ NKT   | Promotion of eosinophilia and neutrophilia | No AHR development (restored by IL-17RB⁺ NKT transfer) | Not tested | 31,32      |
| **Asthma**             |                 |                    |                                           |                        |                                     |                                    |            |
| Ovalbumin model        | CD4⁺ (IL-17RB⁺) NKT | IL-4/IL-5/IL-13 TSLP-mediated IL-33-mediated? | Eosinophilia, neutrophilia, ovalbumin-specific IgE | Alleration of asthma symptoms       | Alleration of AHR due to a repolarization of the immune response toward a T\(_{h1}\)-profile (intraperitoneal, intranasal, intravenous) | 98–100,105,107–111 |
| Ovalbumin + HDE        | Undetermined    | IFN-γ and IL-13    | CD1d-dependent                             | Participate in the adjuvant effect of HDE (eosinophilia, neutrophilia and IgE) | Alleration of asthma symptoms       | Not tested | 103        |
| **COPD**               |                 |                    |                                           |                        |                                     |                                    |            |
| Virus-induced (Sendai virus) | CD4⁻ NKT       | IL-13              | CD1d-dependent                             | Activation of IL-13-producing macrophages | Decrease mucus production and goblet-cell metaplasia | Not tested | 97         |
| Cigarette smoke exposure | Undetermined    | IL-17              | To be determined                           | Alteration of lung functions | Not tested                          | Not tested | Pichavant, in press |
| **ALI**                |                 |                    |                                           |                        |                                     |                                    |            |
| Lipopolysaccharide-induced | —               | —                  | —                                         | —                      | No differences with control mice     | Exacerbation of ALI (intraperitoneal) | 129        |
| IC-induced             | Undetermined    | IFN-γ/MIP-1α       | CD1d-dependent                             | Recruitment of inflammatory cells and activation (TNF-α and IL-1β) of alveolar macrophages and DCs | Lower fibrosis and associated-parameters | Exacerbation (intravenous) | 130        |
| Sickle cell disease    | CXCR3⁺ NKT      | IFN-γ              | CD1d-dependent                             | Lymphocyte recruitment | Reverse pulmonary dysfunction (using anti-CD1d mAb) | Not tested | 131,132    |
| **Fibrosis**           |                 |                    |                                           |                        |                                     |                                    |            |
| Bleomycin              | CXCR3⁺ NKT      | IFN-γ              | Not tested                                 | Suppression of TGF-β synthesis | Increased pulmonary fibrosis and mortality | Attenuation of fibrosis (intraperitoneal) | 133–135    |
| Hypersensitivity pneumonitis | Undetermined    | IL-4               | Not tested                                 | Suppression of IFN-γ-producing neutrophils | Exacerbation of HP (suppression restored by transfer of IFN-γ⁻⁻⁻ but not IL-4⁻⁻⁻ NKT) | Not tested | 136        |

Abbreviations: α-GalCer, α-galactosylceramide; AHR, airway hyperreactivity; ALI, acute lung injury; COPD, chronic obstructive pulmonary disease; DCs, dendritic cells; HDE, house dust extract; HP, hypersensitivity pneumonitis; IC, immune complex; IFN, interferon; IL, interleukin; NA, not applicable.
Airway diseases that resemble asthma can be artificially induced by intranasal administration of \( \alpha \)-GalCer or microbial-derived (i.e., *Sphingomonas*) NKT cell activators in mice, pigs, and nonhuman primates.\(^1\)\(^3\)\(^,\)\(^9\)\(^2\)\(^–\)\(^9\)\(^5\) The pathology has features that resemble farmer’s or bird fancier’s lung disease and is due to exposure to organic dusts.\(^1\)\(^3\)\(^,\)\(^9\)\(^2\)\(^–\)\(^9\)\(^5\) Although the production of IL-4/IL-13 is important in AHR development, administration of \( \alpha \)-GalCer also induces IL-17 production by lung CD4\(^+\) NKT cells, which exacerbates neutrophilia and AHR.\(^3\)\(^4\) Exposure to oxidant pollutants such as ozone also promotes airway neutrophilia and AHR upon a phenomenon that depends on IL-17 production by NK1.1\(^+\) NKT cells.\(^9\)\(^6\) Finally, in a model of virus-induced asthma, IL-13-producing NKT cells have a part in AHR development and lung injury.\(^9\)\(^7\)

Through the development of a strong IL-4/IL-13 axis, NKT cells have a critical role in causing experimental allergic asthma,\(^9\)\(^8\)\(^–\)\(^1\)\(^0\) although this has been called into question.\(^1\)\(^1\) Airborne exposure to microbial products is clearly linked to asthma in humans\(^1\)\(^0\)\(^2\)\(^–\)\(^1\)\(^0\) and most aeroallergens are contaminated with microbial products (such as lipopolysaccharide) that can promote allergic airway inflammation in experimental systems. Whether naturally occurring substances present in the environment might serve as triggers of airway inflammation through direct NKT cell activation has been examined. Asthma reaction can be induced by respiratory coadministration of ovalbumin and bacterial lipids known to activate NKT cells or with \( \alpha \)-GalCer.\(^1\)\(^3\) It was also reported that house dust extracts contain lipid Ags that activate NKT cells independently of the TLR signaling pathway.\(^1\)\(^0\)\(^3\) Lipids from plants, and not only from microorganisms, might also contribute to asthma. For example, pollens contain phospholipids able to bind CD1d and activate human NKT cells.\(^1\)\(^0\)\(^4\) Thus, certain environmental factors, by indirectly (lipopolysaccharide) and/or directly (lipid Ags) activating NKT cells, might promote sensitization to conventional respiratory allergens.

In conclusion, NKT cells have a role in promoting allergic asthma by inducing AHR (in synergy or not with Th2 cells) and, through their adjuvant properties, by providing help to the development of pathogenic allergen-specific Th2 lymphocytes. Another mechanism has been proposed in which NKT cells are targeted by some Th2-associated factors to exacerbate asthma. Thus, NKT cells seem required for the deleterious effects of IL-25 and thymic stromal lymphoprotein, two important factors produced by Th2 cells, mast cells, basophils, and/or epithelial cells and involved in asthma initiation and amplification. The Th2-promoting and asthma-triggered effects of IL-25 is due to CD4\(^+\) NKT cells expressing IL-17RB.\(^3\)\(^1\)\(^,\)\(^3\)\(^2\) In response to IL-25, this peculiar NKT cell subset produces IL-13 to amplify asthma reaction. Of note, NKT cells also produce IL-13 in response to thymic stromal lymphoprotein.\(^1\)\(^0\)\(^5\) Finally, IL-33 might also exacerbate asthma symptoms by promoting the release of Th2-type cytokines by NKT cells.\(^1\)\(^0\)\(^6\)\(^,\)\(^1\)\(^0\)\(^7\) Attempts to harness NKT cell activities in asthma have been performed as treatment of ovalbumin-sensitized animals at the time of Ag challenge with \( \alpha \)-GalCer,\(^1\)\(^0\)\(^8\)\(^–\)\(^1\)\(^1\)\(^1\) \( \beta \)-GlcCer (a naturally occurring lipid),\(^1\)\(^1\)\(^2\) or R848 (a TLR7 agonist)\(^1\)\(^1\)\(^3\) alleviated experimental asthma through IFN-\( \gamma \) and/or IL-27 production. Prophylactic treatment of 2-week-old mice with some NKT cell agonists including a cholesterol-derived lipid from *Helicobacter pylori* can also protect against asthma, probably by inducing local expansion of a subset Th1-biased NKT cells.\(^1\)\(^1\)\(^4\)

Of particular interest, interaction between endogenous flora and NKT cells during early life seems to be a cardinal factor to prevent uncontrolled inflammatory responses in adults such as asthma.\(^1\)\(^0\) Thus, mice bred under germ-free conditions show an accumulation of mucosal (Th2 bias) NKT cells via a CXCL16-dependent mechanism. Interestingly, germ-free mice developed an allergic airway response significantly greater compared with specific pathogen-free mice, and this effect was CD1d dependent.\(^1\)\(^0\)

As NKT cell agonists are likely to be ubiquitous (soil, air, aqueous environment),\(^1\)\(^0\)\(^3\) it seems probable that repeated exposure to natural aerosols containing lipids might have significant pathological implications in humans. Although convergent studies demonstrate that NKT cells have a role in asthma in animal experimental models, the evidence in human subjects is still equivocal.\(^1\)\(^1\)\(^5\)\(^–\)\(^1\)\(^1\)\(^7\) An original study reported an abundance of NKT cells in the Airways (\( \sim 60\% \) of CD4\(^+\) T-cells) of patients with asthma,\(^1\)\(^1\)\(^8\) but all other studies reported mild, if any, differences compared with controls.\(^1\)\(^4\),\(^1\)\(^5\),\(^1\)\(^1\)\(^9\)–\(^1\)\(^2\)\(^5\)

**Chronic obstructive pulmonary disease**

COPD is characterized by a chronic inflammation of the airways leading to airflow limitation. COPD has increased in frequency and severity in developed nations and now affects more than 200 million people (the fourth leading cause of death worldwide).\(^1\)\(^2\)\(^6\),\(^1\)\(^2\)\(^7\) Cigarette smoke and industrial pollutant exposure are the most important risks factors for COPD, and the imbalance between oxidants and antioxidants strongly contributes to the pathology.\(^1\)\(^2\)\(^8\) Patients developing COPD have an increased number of NKT cells in the lungs,\(^9\)\(^7\) although in a previous report no differences were found.\(^1\)\(^5\) Using an experimental model of chronic lung disease induced by Sendai virus infection, it was shown that NKT cells stimulate macrophages to produce IL-13, thus stimulating the development of goblet-cell metaplasia, AHR, and mucus production, independently from the adaptive immune response.\(^9\)\(^7\) More recently, using a model of chronic exposure to cigarette smoke mimicking COPD symptoms, we have demonstrated the requirement of NKT cells in altered lung functions and emphysema, a key feature of COPD (Pichavant et al., in press). In this setting, it is likely that oxidative stress induced by cigarette smoke initiates an activation pathway in sentinel cells resulting in the stimulation of NKT cells and thereby lung dysfunctions. Thus, NKT cells might be involved in COPD, although further studies are clearly necessary to establish the relevance in the human system.
Acute lung injury and lung fibrosis
NKT cells are detrimental in models of immune complex- and lipopolysaccharide-induced acute lung injury.\textsuperscript{129,130} In these settings, production of IFN-\(\gamma\) by NKT cells enhances the local production of chemokines (MIP\(\alpha\)/CCL3), thus amplifying the recruitment of inflammatory cells, such as granulocytes and/or monocytes, and promoting tissue damage. NKT cells are also deleterious during experimental sickle cell disease, a disease caused by mutation of \(\beta\)-globin and characterized by episodes of ischemia-reperfusion injury in the lungs. In this system, NKT cells promote chronic pulmonary inflammation and dysfunction through IFN-\(\gamma\)-induced recruitment of lymphocytes.\textsuperscript{131} In patients with sickle cell disease, NKT cells in blood are increased in absolute number and display an activated phenotype in comparison with healthy controls.\textsuperscript{131} Of interest, treatment of mice with an adenosine 2A receptor agonist reverses pulmonary disease, in part, by inhibiting cytokine production by NKT cells.\textsuperscript{132} As some adenosine 2A receptor agonist(s) are clinically approved in humans (e.g., to treat coronary disease), this might represent a general strategy to treat diseases associated with NKT cell activation.

As reviewed above, NKT cells are usually deleterious during lung inflammation. However, there are few examples suggesting that they can have a protective role. Pulmonary fibrosis is a progressive illness characterized by interstitial fibrosis. The potential role of NKT cells in acute pulmonary fibrosis has been studied using bleomycin. In this system, inflammatory cell infiltration and lung collagen deposition are the hallmarks of the pathology. Treatment with \(\alpha\)-GalCer attenuated the development of bleomycin-induced pulmonary fibrosis.\textsuperscript{133} Moreover, endogenous activation of NKT cells during bleomycin treatment also resulted in lower pathogenesis, although the mode of NKT cell activation in this system has not been characterized. Whatever the mechanisms, the antifibrotic role of NKT cells in pulmonary fibrosis was associated with IFN-\(\gamma\) production and downregulation of TGF-\(\beta\)1 synthesis.\textsuperscript{134,135} The potential role of NKT cells in chronic fibrosis is still unknown and no data exist to predict a role for NKT cells in human lung fibrosis. Finally, the production of IL-4 by NKT cells protects against hypersensitivity pneumonitis by suppressing IFN-\(\gamma\)-producing neutrophils and CD8\(^+\) T cells.\textsuperscript{136}

NKT CELLS DURING PULMONARY INFECTIONS
A growing body of evidence documents a critical role for NKT cells in host defense against respiratory pathogens. On the other hand, they can inadvertently cause tissue damage associated with ongoing inflammation and, in some circumstances, control the runaway inflammation (Table 3).

Viruses
Pulmonary NKT cells contribute to pathogen clearance in acute viral infections, but this might be at the expense of uncontrolled inflammation.\textsuperscript{50} This role in immune surveillance against viruses is partly based on their capacity to mature DCs and subsequently activate potent cytotoxic NK cells and CD8\(^+\) T lymphocytes. For instance, endogenous or exogenous (\(\alpha\)-GalCer) activation of lung NKT cells leads to antiviral CD8\(^+\) T-lymphocyte response and virus clearance during primary respiratory syncytial virus infection.\textsuperscript{137} Bronchiolitis is a feature of respiratory syncytial virus infection in young children. Through Th2-type cytokines, NKT cells might lead to pulmonary eosinophilia and fibrosis after respiratory syncytial virus infection, although this has not yet been studied in models of neonatal respiratory syncytial virus infection.

Influenza A virus (IAV) infection is one of the most important causes of respiratory tract diseases.\textsuperscript{138,139} Infection with IAV induces severe pulmonary immune pathology that leads to substantial human morbidity and mortality every winter. Further, IAV infection can enhance susceptibility to secondary bacterial infections, leading to increased lung pathogenesis and sepsis.\textsuperscript{140,141} In the mouse system, exogenous activation of the lung NKT cells by \(\alpha\)-GalCer protects against lethal H1N1 and H3N2 influenza in prophylactic settings (refs 142,143 and our unpublished data). When combined with inactivated viruses or IAV Ags, \(\alpha\)-GalCer exerts potent adjuvant properties to amplify antibody and CD8\(^+\) T-lymphocyte responses able to neutralize IAV.\textsuperscript{149,142,144–148}

The natural, endogenous, role of NKT cells has been examined during experimental influenza infection (Figure 2). There is a consensus that NKT deficiency resulted in worse inflammation, albeit through different mechanisms according to experimental conditions.\textsuperscript{143,149–151} Using a H1N1 strain, De Santo et al.\textsuperscript{149} suggested that the increased pathology observed in NKT-deficient animals was due to an enhanced virus spread. The authors proposed that NKT cells control IAV replication by blunting the immunosuppressive activity of myeloid-derived suppressive cells, a population that prevents specific CD8\(^+\) T-cell response. Using a highly inflammatory H3N2 strain, we have confirmed the role of NKT cells in CD8\(^+\) T-cell response, a phenomenon rather due to their ability to induce DC maturation/migration to the draining lymph nodes.\textsuperscript{151} Moreover, NKT cells regulate the strength of NK cell activation,\textsuperscript{151} a process that might be beneficial regarding the role of NK cells in IAV-associated pathogenesis.\textsuperscript{152,153} Kok et al.\textsuperscript{143} demonstrated that NKT cells control the recruitment of pulmonary inflammatory monocytes, a cell population that strongly contributes to IAV-associated pathogenesis.\textsuperscript{154} On the other hand, we proposed that the protective role of NKT cells on the control of pneumonia might be linked to their ability to produce IL-22.\textsuperscript{37} This cytokine protects airway epithelium against cell damage caused by IAV\textsuperscript{37} and, through this unique property, strengthens the epithelial barrier integrity to limit excessive lung inflammation.\textsuperscript{37,155–157} This later effect might be crucial to limit bacterial superinfection after influenza.\textsuperscript{155} Of interest, IAV infection of suckling mice leads to expansion of regulatory CD4\(^+\) NKT cells that protect against lung Th2-mediated damages.\textsuperscript{114} To conclude, NKT cells have multiple beneficial roles during experimental IAV infection, a property that is likely attributed to diverse NKT cell subsets. Whether or not...
NKT cells have a role during human influenza and whether they have a different impact according to the pathogenicity index of IAV, await future studies.

### Bacteria and mycobacteria

The extracellular, Gram-positive bacterium *S. pneumoniae* (also called pneumococcus) is a serious human pathogen that...
causes severe lung and/or invasive disease. The natural positive role of NKT cells in the control of *S. pneumoniae* infection has been largely described, although the mechanisms involved are still elusive. We and others have shown that intranasal administration of α-GalCer can prophylactically protect against lethal *S. pneumoniae* infection. Analysis of mechanisms involved revealed that IFN-γ and IL-17, as well as neutrophils, are necessary to clear *S. pneumoniae*. Although this is controversial, endogenous and exogenous activation of the lung NKT cells also lead to the control of *Pseudomonas aeruginosa*, a Gram-negative bacterium that causes major problems in immunocompromised individuals including patients with cystic fibrosis or other obstructive lung disorders. *Staphylococcus aureus* remains a common cause of nosocomial bacterial infections and are often antibiotic resistant. The natural role of NKT cells during pulmonary *S. aureus* infection is still unknown. However, it was reported that, after intranasal administration, *Staphylococcus* enterotoxin B acts as a super Ag to initiate NKT cell activation, which in turn triggers acute lung injury. However, when injected via the systemic route, NKT cells have a minor effect on super Ag-induced shock. Of interest, in both settings, NKT cell activation occurs in a CD1d-independent manner but through major histocompatibility complex class 2/NKT TCR interaction. NKT cells have a protective or harmful role during infection with the intracellular bacteria *Chlamydia*, depending on the species studied. It is possible that the different organisms elicit activation of distinct subsets of NKT cells to promote either a protective Th1 response (*C. pneumonia*) or a detrimental Th2 response (*C. muridarum*). NKT cells might also be detrimental during *Legionella pneumophila* infection.

The role of NKT cells during *Mycobacterium* infection has been extensively studied both in animal models and in humans. It is estimated that one-third of the world’s population is infected with *M. tuberculosis* (~2 million deaths per year). There is evidence supporting the role of NKT cells in murine tuberculosis infection, although their absence is not essential to control the infection. By contrast, exogenous activation of NKT cells with α-GalCer protected susceptible mice from tuberculosis. Moreover, early NKT cell activation has a role in resistance to *M. bovis* Bacillus Calmette–Guerin infection in mice presumably through an IFN-γ-dependent pathway. Of interest, NKT cell deficiency in humans might be crucial for the development of active/acute tuberculosis. These patients have less peripheral NKT cells compared with patients with latent tuberculosis, and normal NKT cell frequencies can be restored by treatment for active tuberculosis.

**Fungi**

Fungi are widely distributed in nature causing human illnesses, including fatal invasive disease, especially in immune-compromised (e.g., acquired immunodeficiency syndrome, cancer) patients. Experimental models demonstrated that NKT cells have a positive role in the control of fungal infections including *Cryptococcus neoformans*, an opportunistic yeast-like fungus. NKT cells accumulate in the lungs in chemokine

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**Figure 2** Versatile role of type 1 natural killer T (NKT) cells during experimental influenza A virus (IAV) infection.
Mucosal sites, including the lung, might offer a specific environment and pathogens) and plant-derived Ags should be encouraged. The role of NKT cells during Candida (e.g., C. albicans) and Pneumocystis species pulmonary infections, which can also cause serious problems in immune-compromised individuals, is still ignored and worthy of future studies.

CONCLUSIONS

There has been significant progress in our knowledge about the modalities of activation and functions of NKT cells but many unanswered questions remain. As innate-driven NKT cell activation has a major influence on the strength and nature of immune responses, analysis of danger signals associated with sterile insults and infections must be further analyzed. This is important because according to the nature of the initial trigger, the way in which NKT cells are activated and influence the pathology might be different. What is the contribution of endogenous/exogenous lipid Ags vs. cytokines, or other host factors, on NKT cell activation and cytokine profiles? Research toward the identification of novel self-, microbial- (commensals and pathogens) and plant-derived Ags should be encouraged. The tissue where NKT cells are activated is also important and mucosal sites, including the lung, might offer a specific environment affecting the activation and functions of NKT cells.

There is also an urgent need to precisely define the NKT cell subset composition and functions at mucosal sites. Although NKT cells can modulate disease outcomes through the release of Th1-, Th2-, and Th17-related cytokines, it is also possible that regulatory NKT cells in the pulmonary mucosa have a crucial role, for instance, by controlling the production of harmful cytokines. A better characterization and knowledge of these regulatory cell subtypes will be an important breakthrough in the field.

As reviewed above, there is a consensus view about the significance of NKT cells in pulmonary disorders and respiratory infections in experimental models. The situation in humans is not so clear, as clinical information is sparse and contradictory data regarding the frequency and functions of NKT cells in patients have been reported. To establish a role for NKT cells in pulmonary diseases, and eventually the significance of NKT cells in prognosis and diagnosis, clinical studies are clearly warranted. It is expected that the use of more stringent criteria to analyze NKT cell subsets, larger cohorts, and a better definition of the clinical cases being analyzed will bring valuable information in the field.

Could NKT cells provide attractive therapeutic targets for treating acute or chronic inflammatory pathologies that affect the lungs and/or respiratory infections, and, if so, how can we progress toward the development of more effective treatments that target mucosal NKT cells? As mentioned above, an in-depth understanding of immune mechanisms that underlie NKT cell activation and functions is required in order to progress toward developing more effective treatments, for instance, in the design of drugs that block NKT cell functions and/or trafficking or that skew NKT cell functions. Among possible strategies, the use of pro-Th1, pro-Th2, or pro-Th17 NKT cell agonists as well as components that block NKT cell activation might be envisaged. Concerning the latter option, di-palmitoyl-phosphatidylethanolamine polyethylene glycol and GM3 represent interesting therapeutic candidates in NKT cell-mediated diseases as they bind to CD1d with high affinity without inducing NKT cell activation.100,185

There are many situations in which immune suppression arises and so NKT cell-based assistance of immunity to control respiratory infections might represent useful alternatives. Acute pulmonary viral infection such as influenza leads to secondary bacterial (e.g., S. pneumoniae) infection with dramatic clinical consequences. Chronic viral infections such as human immunodeficiency viruses or herpes viruses are associated with enhanced frequencies of respiratory infections (bacteria and fungi) as in some cancers. In these clinical situations, and in certain circumstances, the use of α-GalCer or α-GalCer derivatives might prevent respiratory infections.

Of importance, optimal NKT cell-based immunotherapy will not be reached without considering the diversified, and somehow opposing, functions of NKT cell subsets and, to a larger extent, of other CD1-restricted cells present in the lungs. In parallel to approaches based on classical experimental models, the use of humanized mice and nonhuman primates should be highly informative not only to better understand the role of NKT cells in pulmonary diseases but also for the design and application of preventive or curative NKT cell-based immunotherapies.

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DISCLOSURE

The authors declared no conflict of interest.

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