Natural killer T cells in pulmonary disorders

Matija Rijaveca, Sinisa Volarevicb, Katarina Osolnika, Mitja Kosnik,a, Peter Korosec,a

a University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia
b Department of Molecular Medicine and Biotechnology, School of Medicine, University of Rijeka, Croatia

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Summary
Natural killer T (NKT) cells, a unique subgroup of lymphocytes with features of both T and natural killer (NK) cells, represent a bridge between innate and adaptive immunity. They have the ability to either promote or suppress immune responses. With these immunoregulatory functions, NKT cells have emerged as an important subset of lymphocytes with a protective role in some disorders, such as infections, cancer, and possibly sarcoidosis, and a pathogenic role in others, such as asthma, chronic obstructive pulmonary disease and hypersensitivity pneumonitis. Immunotherapeutic interventions to modulate the immune response by targeting iNKT cell functions has become a challenging field and has shown promising results for the development of new therapies.

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NKT cell heterogeneity

Natural killer T (NKT) cells and NKT-like cells comprise a unique, yet highly heterogeneous subgroup of lymphocytes that express features of both T cells and natural killer (NK) cells and coexpress T-cell receptors (TCRs) together with markers associated with NK cells, such as CD56 and/or CD161.1-3 These cells are phenotypically and functionally highly diverse, but all are hybrid by nature and represent a bridge between innate and adaptive immunity (Table 1). Along with macrophages, dendritic cells, and others, NK cells are part of the innate immune system, whereas the acquired immune system is defined by the clonal expansion of antigen-specific T and B cells and their memory immune responses. Because NKT and NKT-like cells are part of both systems, their absence, depletion, or improper function affects both innate and acquired immunity.1-4

Type I and Type II NKT cells

In humans there are two major subsets of NKT cells that are dependent on the presentation of the antigen through CD1d, a nonpolymorphic class I antigen-presenting molecule. CD1d is expressed on many different cells, including dendritic cells, thymocytes, B cells, monocytes, and macrophages. When glycolipid antigen binds to CD1d, the antigen/CD1d complex binds to the TCR of NKT cells.2,5 The most widely studied are CD1d-dependent cells, which express a highly restricted TCR with an invariant Vα24-Jα18 preferentially paired with Vβ11. These are called invariant NKT (iNKT) cells or type I NKT cells.1,2,4,6 The best-known iNKT cell antigen is the glycolipid α-galactosylceramide (α-GalCer), originally isolated from the marine sponge Agelas mauritianus, which is also used to identify cells as α-GalCer-loaded CD1d tetramers.1,7 Other glycolipid antigens for iNKT cells have been identified, including bacterial glycolipids such as α-galacturonosylceramide, α-glucuronosylceramide, and α-galactosyl-diacylglycerol, as well as mammalian glycolipids such as isoglobotrihexosylceramide (iGb3) and the disialoganglioside GD3.1,4-9 Activated iNKT cells rapidly release large amounts of both T-helper 1 (Th1), Th2, and/or Th17-specific cytokines and exhibit both antigen-specific and NK-like cytolytic activities.1,2,5,10 Invariant NKT cells are therefore able to both promote and suppress immune responses, presumably due to the existence of many distinct subsets with different functions.1,2,4-6 Among these, CD4+ iNKT cells produce both Th1 (such as interferon-γ (IFN-γ)) and tumor necrosis factor α (TNF-α)) and Th2 cytokines (such as interleukin-4 (IL-4), IL-10, and IL-13), and are associated with Th10-type immune responses, whereas CD4–CD8– iNKT cells are similar and mainly produce Th1 cytokines.1,2,11,12 Furthermore, a subset of iNKT cells that produces high amounts of cytokine IL-17 was recently
Table 1: Classification of NKT and NKT-like cells

<table>
<thead>
<tr>
<th>Antigen presentation</th>
<th>Invariant NKT cells</th>
<th>Type II NKT cells</th>
<th>NKT-like cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD1d</td>
<td>CD1d</td>
<td>MHC</td>
</tr>
<tr>
<td></td>
<td>Glycolipid</td>
<td>Hydrophobic &amp; aromatic molecules</td>
<td>Peptide</td>
</tr>
<tr>
<td>TCR repertoire</td>
<td>Vα24-Jα18, Vγ11</td>
<td>Diverse</td>
<td>Diverse</td>
</tr>
<tr>
<td>NK cell markers</td>
<td>CD161+/−, CD56+/−</td>
<td>CD161+/−, CD56+/−</td>
<td>CD161 and/or CD56</td>
</tr>
</tbody>
</table>

Table 2: NKT and NKT-like cells in pulmonary disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>Altered numbers of iNKT cells (inconsistent results)</td>
</tr>
<tr>
<td>COPD</td>
<td>Increased numbers of INKT and CD3+CD56+ NKT-like cells in lungs, deficiency of CD3+CD56+ NKT-like cells in peripheral blood</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>Deficiency of iNKT cells</td>
</tr>
<tr>
<td>Hypersensitivity pneumonitis</td>
<td>Increased numbers of CD3+CD16/CD56+ NKT-like cells, normal numbers of INKT cells</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>Deficiency and impaired function of INKT cells</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>Deficiency of INKT cells in active phase</td>
</tr>
</tbody>
</table>

identified.2,12–14 These iNKT cells are CD4 NK1.1+, which distinguishes them from the majority of other NKT cells and may have an important role in IL-17-mediated disease.2,13

The second group of CD1d-dependent cells, called also type II NKT cells, expresses an unbiased TCR repertoire and recognizes several hydrophobic antigens such as sulfatide, lysophosphatidylcholine, and aromatic molecules.2,15,16 These cells have been less thoroughly studied, mainly because they cannot be identified using α-GalCer-loaded CD1d tetramers. They are not discussed further in this review.

NKT-like cells

The third group that resembles NKT cells, and is often referred to as NKT cells, are so-called NKT-like cells, or CD1d-independent T cells. These cells are independent of CD1d for their activity,3,6,10 and so they depend on the presentation of antigen to conventional MHC class I and II molecules and express unbiased α/β T-cell receptors. Other than the expression of CD161 and/or CD56, there is little evidence that these NKT-like cells are related to NKT cells.3,17 NKT-like cells comprise a highly heterogeneous group of cells, being either CD4+, CD8+, or double-negative CD4−CD8− T cells that also express NK-cell markers such as CD161 and/or CD56.3

Role of NKT cells in various disorders

The roles of different subsets of NKT cells in humans are not completely understood, but it is well established that many have immunosuppressive activity and/or may promote enhanced cell-mediated immunity.18,19 NKT cells have been implicated in several biological processes, including cell-mediated suppression of tissue destruction, anti-tumor responses, autoimmunity, host defense, allergy, and inflammation, as well as direct influences on B-cell proliferation and antibody production.1–3,20,21 Their role has mainly been associated with priming and regulating the immune response because numerical and/or functional impairments have been reported in many pulmonary disorders. Thus, NKT and NKT-like cells might play a critical role in several pulmonary diseases, including asthma, chronic obstructive pulmonary disease (COPD), interstitial lung diseases such as sarcoidosis and hypersensitivity pneumonitis (HP), lung cancer, and infectious diseases such as tuberculosis (Table 2).1–3

Asthma

Invariant NKT cells produce large amounts of both Th1 and Th2 cytokines and may either inhibit or exacerbate allergic response. Because asthma is characterized by a highly polarized Th2 immune response, tissue inflammation, and airway hyperreactivity, various immune system cells, including NKT cells, have important roles in it. However, the reported results regarding the contribution of iNKT cells in asthma have been controversial. Early reports demonstrated an increased number of iNKT cells in the lungs of patients with asthma compared to patients with sarcoidosis and healthy controls.22,24 However, in subsequent years these findings were challenged by other groups, who reported lower iNKT cell counts than previously reported.25,26 Recent publications have highlighted the role of iNKT cells in asthma because several studies have shown a significant increase of iNKT cells in patients with asthma.27,28 Furthermore, it has been demonstrated that iNKT cells play an important role in the development of airway hyperreactivity and airway inflammation in mice and nonhuman primates,22,27 in which several environmental substances, including bacterial glycolipids, were shown to activate iNKT cells.29 It is
now becoming evident that iNKT cells are involved in the pathogenesis of asthma because they might be employed as effectors as well as amplifiers of T<sub>H</sub>2 cell response.\textsuperscript{30,31}

**Chronic obstructive pulmonary disease**

COPD is a disease consisting of emphysema, respiratory bronchiolitis and chronic bronchitis that is largely driven by T<sub>H</sub>1-mediated immune responses.\textsuperscript{32,33} Increased levels of iNKT cells were found in the lung tissue of patients with COPD. Furthermore, interactions of CD4<sup>+</sup> iNKT cells with macrophages, subsequent activation and IL-13 production appears to be crucial for the development of chronic airway disease in animal mouse model.\textsuperscript{34} Beside that increased numbers as well as enhanced cytotoxicity of CD3<sup>+</sup>CD56<sup>+</sup> NKT-like cells in the induced sputum of COPD patients was observed, what is in contrary to what was observed in the peripheral blood. Therefore, those cells could be selectively recruited to the lungs.\textsuperscript{35,36} Furthermore, the proportion of CD8<sup>+</sup> NKT-like cells was increased and CD4<sup>+</sup> NKT-like cells were decreased in COPD patients. Therefore, CD8<sup>+</sup> NKT-like cells with the production of predominantly T<sub>H</sub>1-type cytokines could be responsible for the pro-inflammatory immune response in the lungs of COPD patients.\textsuperscript{36}

**Sarcoidosis**

Sarcoidosis is a multi-system disorder of unknown etiology that predominantly involves the lungs and is characterized by T<sub>H</sub>1-biased CD4<sup>+</sup> T cell response and the formation of granulomas. Other than evidence of T-cell involvement in the characteristic immune response, it is still unclear how this response is generated. The immunoregulatory properties of iNKT cells may play a very important role in sarcoidosis because loss of immunoregulation due to a deficiency and/or impaired function of these cells might contribute to the amplified and prolonged T-cell activity that characterizes sarcoidosis.\textsuperscript{37-40} Deficiencies of iNKT cells in the blood and bronchoalveolar lavage fluid (BALF) of sarcoidosis patients have been reported.\textsuperscript{37,38,40} The data on granulomas are inconsistent because there are reports on both the absence of iNKT cells in granulomatous\textsuperscript{38} and cutaneous lesions\textsuperscript{41} and also the accumulation of iNKT cells in granulomatous lesions of sarcoidosis patients.\textsuperscript{37} Furthermore, stimulated peripheral blood iNKT cells from sarcoidosis patients demonstrated impaired IFN-γ production.\textsuperscript{37} A low number of iNKT cells and impaired IFN-γ production might be involved in the inflammatory process in sarcoidosis and disease progression because the involvement of IFN-γ in granuloma formation is well documented.\textsuperscript{37,39} However, longitudinal analysis of iNKT cell frequency and/or function throughout the course of the disease is needed to elucidate the exact role of these cells in the pathogenesis of sarcoidosis.

**Hypersensitivity pneumonitis**

A recent study showed significantly higher CD3<sup>+</sup>CD16/56<sup>+</sup> iNKT-like cell frequencies in the BALF of patients with HP compared to study cases with other interstitial lung diseases.\textsuperscript{42} Therefore, assessing the CD3<sup>+</sup>CD16/56<sup>+</sup> cells in the BALF might be a helpful adjunct in the diagnosis of HP. It was further demonstrated that a large proportion of the BALF cells of HP patients were of the CD8<sup>+</sup>CD56<sup>+</sup> subset, and the majority of these cells did not express the invariant V<sub>α</sub>24 T-cell receptor or CD161. Wajchman et al. showed that this distinct CD8<sup>+</sup>CD56<sup>+</sup>CD161<sup>+</sup> cell phenotype displays both antigen-specific (CTL-like) as well as NK-like cytolytic activities that are independent of HLA class I and CD1 molecules.\textsuperscript{10} Pittet et al. demonstrated that the potent cytolytic effector function of these cells closely correlates with CD56 surface expression and that these cells contain high amounts of intracellular perforin and granzyme B.\textsuperscript{43} The frequencies of iNKT cells in the BALF of HP patients were comparable to those in the BALF of healthy control subjects, and thus iNKT cells are unlikely to play an important role in the pathogenesis of HP.\textsuperscript{25,26,40}

The increased number of CD8<sup>+</sup>CD56<sup>+</sup> cells in the BALF of HP patients is interesting from several points of view. First, animal models suggest that hypersensitivity pneumonitis is characterized by a T<sub>H</sub>1-type response\textsuperscript{44} and that IFN-γ is necessary for the development of disease because IFN-γ knockout mice are resistant to developing hypersensitivity pneumonitis.\textsuperscript{45} Exposure to IL-12 further enhanced IFN-γ production and consequently modulated the severity of experimental hypersensitivity pneumonitis.\textsuperscript{46} Yamasaki et al. demonstrated that clinical hypersensitivity pneumonitis is also characterized by a T<sub>H</sub>1-type response and the predominance of IFN-γ producing T cells.\textsuperscript{47}

Therefore, the potential importance of NKT-like cells in hypersensitivity pneumonitis is strengthened by the findings that CD8<sup>+</sup>CD56<sup>+</sup> cells are more potent inducers of T<sub>H</sub>1-mediated immune response relative to CD8<sup>+</sup> T cells and that the IL-12 stimulated CD8<sup>+</sup>CD56<sup>+</sup> cells can produce much larger amounts of IFN-γ than CD8<sup>+</sup> T cells.\textsuperscript{48} Second, the CD8<sup>+</sup>CD56<sup>+</sup> cells also have a unique chemokine receptor repertoire and a functional migratory response to the CCR5 ligand macrophage inflammatory protein-1 beta (MIP-1β).\textsuperscript{49} MIP-1β can markedly affect the mononuclear infiltration of the lung and was specifically detected in the BALF of HP patients compared to patients with sarcoidosis and cryptogenic fibrosing alveolitis.\textsuperscript{50}

**Lung cancer**

The pro-inflammatory activities of iNKT cells are important for the anti-tumor response in lung cancer. In particular, this includes the ability to induce secondary immune effects by producing INF-γ and subsequently activating the conventional CD8<sup>+</sup> T cells and NK cells.\textsuperscript{51,52} In addition, the perforin-dependent direct cytotoxicity of activated iNKT cells is also important.\textsuperscript{33} Compound action by NKT and NK cells is important for natural anti-tumor immunity.\textsuperscript{53} Decreased numbers and reduced functions of iNKT cells have been reported in peripheral blood of cancer patients as well as in tumor tissues.\textsuperscript{1,51,54} Furthermore, iNKT cells from cancer patients have been shown to have defective cytokine production because they produce less IFN-γ than do iNKT cells from healthy individuals.\textsuperscript{54,55} Activation of iNKT cells also shows promising results in cancer immunotherapy. Administration of α-GalCer and the subsequent activation of iNKT, as well as treatment with in vitro-activated iNKT cells, have demonstrated clear clinical benefits in lung cancer. The therapy was well tolerated and induced an iNKT cell-modulated immune response, prolonging survival time in
non-small cell lung cancer patients.\textsuperscript{56-58} Seventeen patients with advanced non-small cell lung cancer refractory to the standard therapy, were treated with α-GalCer-pulsed autologous PBMCs. Ten patients who displayed increased IFN-γ-producing cells showed significant prolongation of estimated median survival time (31.9 months; range, 14.5 to 36.3 months) as compared with poor-responder patients (9.7 months; range, 3.8 to 25.0 months).\textsuperscript{58}

Infectious diseases: tuberculosis

iNKT cells are able to exhibit both pro-inflammatory and anti-inflammatory properties; they have been implicated in bacterial, viral, fungal, protozoan, and helminthic infections. The immune response to microorganisms can be generated through iNKT cells’ recognition of glycolipid antigens present in pathogens or by indirect activation of iNKT cells in the presence of inflammatory cytokines against microorganisms that do not contain iNKT cell antigens.\textsuperscript{29,59} There is growing evidence that iNKT cell deficiency might be crucial for the development of active tuberculosis in patients infected with \textit{Mycobacterium tuberculosis} because not only was iNKT cell deficiency associated with the active phase of the disease, but normal cell frequencies were also restored by treatment.\textsuperscript{1,60-62} However, further investigations are needed to determine the exact role of iNKT cells in tuberculosis, in addition to determining which subsets and antigens are responsible for activating iNKT cells and which specific cytokines are secreted during the course of disease.\textsuperscript{1,29}

Therapeutic potential

Immunotherapeutic interventions for the modulation of immune response by targeting immunoregulatory functions of iNKT cells has become a challenging field because it is established that these cells might play an important role in a wide range of disorders, including cancer, autoimmune diseases, and infections. Immunotherapeutic approaches targeting iNKT cells should restore proper immune system balance because various subsets and conditions of iNKT cells can either suppress or promote immune responses.\textsuperscript{1,29,59} Activation of iNKT cells by α-GalCer or its analogues has shown promising results in cancer therapy. Activated iNKT cells induced anti-tumor activities of other immune system subsets, such as B and CD8\textsuperscript{+} T cells. Therefore, administration of α-GalCer dendritic cells pulsed with α-GalCer or in vitro-activated iNKT cells are plausible novel cancer immunotherapies.\textsuperscript{56-58} Incorporation of glycolipids that activate iNKT cells, such as α-GalCer or its analogues, into the vaccine would allow us to tailor the immune response to it because it is known that iNKT cells can act as direct effector cells, and may modulate both adaptive and innate immunity.\textsuperscript{1,29} On the other hand, inhibition or blockade of iNKT with anti-CD1d antibodies, or especially a CD1d-dependent antagonist of iNKT cells such as dipalmitoyl-phosphatidyl ethanolamine (DPPE), protected against allergen-induced airway hyperreactivity in a mouse model, demonstrating possible clinical use in treating allergic asthma.\textsuperscript{63} The ability to specifically manipulate iNKT cells raises new possibilities for immunotherapeutic interventions in other diseases in which these cells play important roles, including infections and autoimmune diseases.

Conclusions

iNKT cells represent a bridge between innate and adaptive immunity. With their immunoregulatory functions, they have emerged as an important subset of lymphocytes with a protective role in some diseases, such as infections, cancer, and possibly sarcoidosis, and a pathogenic role in others, such as asthma. Modulation of the immunoregulatory functions of these cells has shown promising results for the development of new therapies.

Conflict of interest statement

The authors declare that they have no competing interest.

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patients independent of tumor type or tumor load. *Int J Cancer* 2005; **116**:87-93.


