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Original article

Role of interferon-γ in Vα14+ natural killer T cell-mediated host defense against *Streptococcus pneumoniae* infection in murine lungs

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Abstract

Previously, we demonstrated that Vα14+ NKT cells and IFN-γ are important upstream components in neutrophil-mediated host defense against infection with *Streptococcus pneumoniae*. In the present study, we extended these findings by elucidating the role of IFN-γ in this Vα14+ NKT cell-promoted process. Administration of recombinant IFN-γ to Jα18KO mice prolonged the shortened survival, promoted the attenuated clearance of bacteria and improved the reduced accumulation of neutrophils and synthesis of MIP-2 and TNF-α in the lungs, in comparison to wild-type (WT) mice. In addition, intravenous transfer of liver mononuclear cells (LMNC) from WT mice into Jα18KO mice resulted in complete recovery of the depleted responses listed above, whereas such effects were not detected when LMNC were obtained from IFN-γKO or Jα18KO mice. Activation of Vα14+ NKT cells by α-galactosylceramide (α-GalCer) significantly enhanced the clearance of bacteria, accumulation of neutrophils and synthesis of MIP-2 and TNF-α in the infected lungs; this effect was significantly inhibited by a neutralizing anti-IFN-γ antibody. Finally, in a flow cytometric analysis, TNF-α synthesis was detected largely by $CD11b^{bright+}$ cells in the infected lungs. Our results demonstrated that IFN-γ plays an important role in the neutrophil-mediated host protective responses against pneumococcal infection promoted by Vα14+ NKT cells.

Keywords: NKT cells; Pneumococcal infection; Neutrophils; IFN-γ; Chemokine; Lung; Mouse

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1. Introduction

Streptococcus pneumoniae is an extracellular Gram-positive bacterium most frequently associated with community-acquired pneumonia, leading to severe pneumonia, bacteremia and meningitis in infants, elderly people and immunocompromised patients [1,2]. Pneumonia caused by this bacterium is characterized by massive infiltration of neutrophils into the alveolar spaces, which are central to the host defense against this infection via an oxygen radical-mediated killing mechanism [3]. The macrophage inflammatory protein (MIP)-2 is a C-X-C chemokine homologous to human interleukin (IL)-8, and plays a major role in attracting neutrophils to sites of inflammation in mice [4,5]. Tumor necrosis factor (TNF)-α is also involved in the accumulation of neutrophils by facilitating their adhesion to vascular endothelial cells through enhancing the expression of certain adhesion molecules [6,7]. These cytokines also act to promote the killing activity of neutrophils against infectious microorganisms [8,9]. In fact, more severe pneumonia occurs when MIP-2 [10,11] and TNF-α [12,13] are suppressed, due to impaired recruitment of inflammatory leukocytes to the infected tissues [4,5,10–13].

Interferon (IFN)-γ has been extensively studied as a mediator of host defenses against intracellular microorganisms [14–17]. Mice with a targeted disruption of the gene for this cytokine are highly prone to infection with such bacteria. In addition, IFN-γ is involved in the host protective responses against extracellular microorganisms [18,19]. Recently, we defined IFN-γ as a critical cytokine in the development of neutrophil-mediated host defense to *S. pneumoniae* infection [20]. IFN-γ promotes the synthesis of MIP-2 and TNF-α and consequently the accumulation of neutrophils in lungs infected with *S. pneumoniae*.

Natural killer T (NKT) cells express both NK cell markers and T cell antigen receptors composed of an invariant α chain with Vα14–Jα18 and a highly skewed β chain with Vβ8.2, Vβ7 and Vβ2. These cells play a regulatory role in the immunological response under various pathological conditions, including tumor formation, autoimmune diseases, allergy and infection, by rapidly secreting large amounts of IFN-γ and IL-4 [21–23]. Previous studies reported the involvement of this particular lymphocyte subset in the host defense against infectious pathogens [24–30]. In our recent study [25], deficiency of Vα14+ NKT cells resulted in increased susceptibility to infection with *S. pneumoniae*, which was associated with reduced synthesis of MIP-2 and TNF-α and an attenuated recruitment of neutrophils into the infected tissues. However, it remains to be understood how the Vα14+ NKT cells function in these responses. The present study was designed to elucidate the role of IFN-γ in this mechanism and present evidence that this cytokine may act downstream of Vα14+ NKT cells in the neutrophil response and host defense against *S. pneumoniae* infection.

2. Materials and methods

2.1. Animals

Vα14+ NKT cell-deficient (Jα18KO) mice were established by targeted deletion of the Jα18 gene segment [31]. IFN-γ gene-disrupted (GKO) mice were established as described previously [32]. These mice were back-crossed more than eight times with C57Bl/6 mice, purchased from Charles River Japan (Osaka, Japan) and used as control WT animals for the KO mice. In some experiments, C57Bl/6 mice were obtained from the Institute for Animal Experimentation, Tohoku University Graduate School of Medicine. Mice were bred in a pathogen-free environment in the Laboratory Animal Center for Biomedical Science, University of the Ryukyus. All mice were used for experiments at 6–15 weeks of age. All experimental protocols described in the present study were approved by the Ethics Review Committee for Animal Experimentation of University of the Ryukyus and Tohoku University.

2.2. Bacteria

A serotype 3, clinical strain of *S. pneumoniae*, designated as URF918, was established from a patient with pneumococcal pneumonia. The bacteria were cultured in Todd–Hewitt broth (Difco, Detroit, MI) at 37 °C in a 5% CO_2 incubator, harvested at 6 h, at mid-log phase of growth, and then washed twice in phosphate-buffered saline (PBS). The inoculum was prepared at $2–6 \times 10^7$ CFU/ml. To induce pulmonary infection, mice were anesthetized by intraperitoneal injection of 70 mg/kg of pentobarbital (Abbott Lab., North Chicago, IL) and restrained on a small board. Live *S. pneumoniae* were inoculated at 50 μl per mouse by insertion of a 24-gauge blunt needle into and parallel to the trachea. In every experiment, 10 times dilution of quantification culture was performed to confirm the inoculation dose.

2.3. Enumeration of viable S. pneumoniae

Mice were sacrificed on day 3 post-infection. Their lungs were carefully dissected and excised, then separately homogenized in 10 ml of half saline by teasing with a stainless-steel mesh at room temperature. The homogenates, appropriately diluted with half saline, were inoculated at 100 μl on 3% sheep blood Muller–Hinton agar plates and cultured for 18 h. The number of subsequent bacterial colonies was recorded.

2.4. Analysis of neutrophils in BAL fluids

Mice were sacrificed 6 h after infection and samples of bronchoalveolar lavage (BAL) fluids were collected as described below. Briefly, after bleeding under anesthesia with ether, the chest was opened and the trachea was cannulated with the outer sheath of a 22-G I.V. catheter/needle unit (Becton Dickinson Vascular Access, Sandy, UT), followed by lavage of the lung three times with 1 ml of chilled PBS. Approximately 1×10^5 cells were centrifuged onto a glass slide at 800 rpm for 3 min using an Auto Smear CF-12D (Sakura Co., Tokyo), and stained using May–Giemsa technique. The total number of neutrophils was estimated by multiplying the total leukocyte number by the proportion of neutrophils in 500 cells.