Host-pathogen interactions during (myco)bacterial respiratory tract infections

Wieland, C.

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Interleukin-1 contributes to an effective clearance of *Mycobacterium kansasii* from the respiratory tract.
Abstract

*Mycobacterium kansasii* is an emerging pathogen that is able to induce pulmonary disease resembling tuberculosis. To determine the role of Interleukin (IL-)1 in lung infection caused by this atypical mycobacterium, IL-1 Receptor type 1 knockout (IL-1R1 KO) and normal wild type mice were intranasally infected with *M. kansasii*. IL-1R1 KO mice demonstrated a reduced bacterial clearance from the lungs and an increased dissemination to the liver, which was accompanied by an enhanced pulmonary inflammatory response. These data identify IL-1 as an important component of the innate immune response to lung infection by *M. kansasii*. 
Introduction

Interleukin (IL)-1 is a potent multifunctional proinflammatory cytokine implicated in various physiological processes and in the pathogenesis of numerous inflammatory diseases (1). Two IL-1 receptors (IL-1R) have been identified: whereas the type II IL-1R functions as a scavenger or decoy receptor, the type I IL-1R is responsible for all IL-1-mediated signalling events (1). M. kansasii is one of the most frequent nontuberculous mycobacterial pathogens isolated from clinical specimens. Infection with M. kansasii can cause pulmonary disease similar to tuberculosis in patients with various immune deficiencies, in particular in subjects with human immunodeficiency virus (HIV) infection or with pre-existing pulmonary disease (2, 3). Since the start of the AIDS epidemic, a vast increase in M. kansasii infection incidence has been observed (4). However, little is known about pathogenicity, mode of transmission and natural reservoir of M. kansasii.

We recently established that CD4 and interferon (IFN)-γ gene deficient mice display a normal resistance against pulmonary infection with M. kansasii, as reflected by a gradual clearance of this pathogen from the respiratory tract with similar kinetics as observed in normal wild type (WT) mice (5). These data suggest that in sharp contrast to host defense against M. tuberculosis, a T helper 1 response does not contribute to protective immunity in M. kansasii infection. We and others recently revealed a protective role of IL-1 during murine tuberculosis, i.e. IL-1R1 KO mice demonstrated increased mycobacterial outgrowth in their lungs and an enhanced mortality after pulmonary infection with M. tuberculosis (6, 7). In light of the apparently different types of protective immune responses during M. tuberculosis and M. kansasii infection, we here determined the role of endogenous IL-1 in host defense against lung infection with the latter species.

Materials and Methods

Mice

All experiments were approved by the Animal Care and Use Committee of the University of Amsterdam. Six to 8 week old IL-1R1 KO (backcrossed 6 times to a C57BL/6 background; kindly provided by Immunex Corporation, Seatle, WA) and C57BL/6 WT mice (sex and age matched; Harlan Sprague Dawley Inc., Horst, The Netherlands) were used.

Experimental infection

M. kansasii, a clinical isolate obtained from a coal -mine worker presenting with tuberculosis-like disease, was grown for 2 weeks in liquid dubos medium containing 0.01% Tween. A replicate culture was incubated at 37°C, harvested at mid-log phase, and stored in aliquots at -70°C. For each experiment, a vial was thawed and
washed with sterile 0.9% NaCl. Briefly, mice were anesthetized by inhalation with isoflurane (Abott Laboratories LTD., Kent, United Kingdom) and mice were intranasally infected with 5x10⁴ CFU of *M. kansasii* as described previously (5). At time-points indicated, 8 mice per group were sacrificed; lungs and liver were removed aseptically, homogenized in sterile saline, plated on Middlebrook 7H11 agar plates to determine bacterial loads. Colonies were counted after 14 to 18 days at 37°C.

**Cytokine and chemokine measurements**
For cytokine measurements, lung homogenates were diluted 1:2 in lysis buffer containing 300 mM NaCl, 30 mM Tris, 2 mM MgCl₂, 2 mM CaCl₂, 1% Triton X-100, and Pepstatin A, Leupeptin and Aprotinin (all 20 ng/ml; pH 7.4) and incubated on ice for 30 min. Homogenates were centrifuged at 1500 x g at 4°C for 15 min; supernatants were sterilized using a 0.22 μm filter (Corning Incorporated, Corning, NY) and stored at -20°C until assays were performed. IL-1β, Tumor Necrosis Factor (TNF), IL-6, cytokine-induced neutrophil chemotactant (KC), Macrophage Inflammatory Protein (MIP)-2, IFNγ and IL-4 were measured using specific enzyme-linked immunosorbent assays using high binding ELISA plates (Greiner Bio-One, Frieckenhausen, Germany) according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

**Histology**
Lungs for histology were harvested after infection, fixed in 10% buffered formaline and embedded in paraffin. Four μm sections were stained with hematoxilin and eosin, and analyzed by a pathologist who was blinded for groups. To score lung inflammation and damage, the entire lung surface was analyzed with respect to the following parameters: interstitial inflammation, endothelialitis, bronchitis, granuloma formation and pleuritis. Each parameter was graded on a scale of 0 to 4, with 0: absent, 1: mild, 2: moderate, 3: severe, 4: very severe. The total “lung inflammation score” was expressed as the sum of the scores for each parameter, the maximum being 20 (5).

**Characterization of inflammatory infiltrates in the lungs**
Pulmonary cell suspensions were obtained by crushing lungs through a 40-μm cell strainer (Becton Dickinson, Franklin Lakes, NJ) as described previously (5). Erythrocytes were lysed with ice-cold isotonic NH⁴Cl solution, washed and counted. Lung cell suspensions obtained from infected mice were analyzed by flow cytometry using FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA) (5). Immunostaining for cell surface molecules was performed for 30 minutes at 4°C using directly labeled antibodies against CD3, CD4, CD8, CD69, Mac-1 and Gr-1 (all
Pharmingen, San Diego, CA). T cell surface molecules were analyzed within the lymphocyte gate containing CD3+ cells, monocytes/ macrophages and granulocytes were analyzed using Gr-1/Mac-1 expression pattern (8).

Statistical analysis
All values are expressed as mean ± SEM unless indicated otherwise. Comparisons were performed with Mann-Whitney U tests using GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA). Values of P<0.05 were considered statistically significant.

Results
Reduced clearance of M. kansasii from the lungs of IL-1R1 KO mice
Earlier studies revealed that during a 20-week observation period after pulmonary infection, the number of M. kansasii CFU gradually decreased in the lungs of WT mice (5). Here we found that also IL-1R1 KO mice displayed decreasing numbers of M. kansasii in lung homogenates during the course of experimentally induced pulmonary

Increased pulmonary inflammation in IL-1R1 KO mice
In M. tuberculosis infection, IL-1 deficient mice exhibited a disturbed formation of granuloma with prominent neutrophil infiltration but reduced migration of lympho-
cytes and mononuclear phagocytes (6, 7). During M. kansasii infection, we found that both in WT and IL-1R1 KO mice the pulmonary infiltrate consisted mainly of mononuclear cells (lymphocytes and monocytes/ macrophages) and to a lesser

Figure 2: Enhanced inflammation and infiltration in lungs of IL-1R, KO mice. (A-D) Representative lung histology and inflammation scores of WT (A and C) and IL-1R1 KO mice (B and D) mice 4 (A and B) and 8 (C and D) weeks after i.n. infection with 5x10^6 CFU of M. kansasii showing enhanced interstitial inflammation and endothelialitis in IL-1R1 KO mice compared to WT mice. H&E staining, magnification x 10. Insets show inflammation scores (for calculation see Methods). (E, F) Total leukocyte counts in lungs of WT and IL-1R1 KO mice 4 (E) and 8 (F) weeks after intranasal infection with 5x10^6 CFU M. kansasii. Different counts were determined using FACS analysis. (G, H) Pulmonary levels of IL-1β (G) and TNF (H) 8 weeks after intranasal infection with 5x10^6 CFU of M. kansasii. All data are representative for 8 animals per group per time point and shown as mean ± SEM. Comparisons between groups were performed using Mann-Whitney U test. *P<0.05 and **P<0.01 versus WT mice.
extent of neutrophils (fig. 2E-F); no concise granulomas were formed and histopathology revealed mainly low grade diffuse interstitial inflammation and pleuritis in the lungs of all mice (fig. 2A-D). Of note, IL-1R1 KO mice demonstrated more extensive lung inflammation, as quantified by the inflammation scores and further documented by higher total cell counts, in particular at 8 weeks post infection (fig. 2). Herein, lymphocyte and neutrophil numbers in whole lung cell suspensions were higher in IL-1R1 KO mice (fig. 2F). No differences in the recruitment of the CD4⁺ and CD8⁺ T cell subsets were detected between WT and IL-1R1 KO mice at 4 and 8 weeks after infection (data not shown). Of note, CD4⁺ T cells in the lung of IL-1R1 KO mice were more activated at 8 weeks post-infection as demonstrated by increased expression of the early activation marker CD69 (1.0±0.2 % for WT CD3⁺/CD4⁺ lymphocytes and 6.0±2.0 for IL-1R1 KO CD3⁺/CD4⁺ lymphocytes; P<0.01). Similarly, IL-1R1 KO mice displayed significantly higher pulmonary TNF and IL-1β levels at 8 weeks after infection (fig. 2G, H). The levels of IFNγ, IL-4, IL-6, MIP-2 and KC did not differ between the two mouse strains (data not shown).

Discussion

Although M. kansasii has emerged as an important pathogen in immunocompromised patients, little is known about its pathogenicity. Immunocompromised and in particular HIV infected patients are susceptible to develop clinical disease after infection with M. kansasii. An increase in M. kansasii disease has been observed because of the onset of the AIDS epidemic (4). IL-1 has been implicated as a regulator of the T helper 1 response to infection with intracellular pathogens (6, 9). Indeed, IL-1R1 KO mice have been found to mount a defective T helper 1 response in models of cutaneous leishmaniasis and pulmonary tuberculosis (6, 9). We here demonstrate that IL-1R1 KO mice have a reduced ability to clear M. kansasii from their respiratory tract. Considering that CD4⁺ T cells and IFNγ do not play a significant role in the protective immunity against M. kansasii in this model (5), our data clearly indicate that IL-1 impacts on host defense by other yet unidentified mechanisms. It is likely that activated resident cells like alveolar and interstitial macrophages as well as epithelial cells produce cytokines like IL-1α or β and chemokines that attract and activate other inflammatory cells and T cells. Moreover, it is conceivable that innate immune responses mediated by Toll-like receptors, complement and antibodies evoke a response via IL-1. Possibly, in the presence of IL-1 signaling, macrophages, monocytes and neutrophils are sufficiently activated to phagocytose and kill M. kansasii bacilli and thus clear the infection. Of note, we recently demonstrated that Toll-like receptor 2 is important for the effective clearance of the non-pathogenic M. smegmatis from mouse lungs (10). Herein, we add evidence to the
notion that local innate host response and innate clearance mechanism in the absence of a strong local and systemic IFN-γ driven adaptive immune system are sufficient to eliminate pulmonary infection with this virulent strain of M. kansasii. IL-1R1 KO mice demonstrated an increased inflammatory response in their lungs as reflected by histopathology and quantitative analysis of leukocytes in whole lung cell suspensions. Likely, this enhanced pulmonary inflammation in IL-1R1 KO mice can be explained by the presence of a higher bacterial load in the lungs of these animals, providing a more potent inflammatory stimulus. Similarly, IL-1R1 KO mice displayed exaggerated lung inflammation after infection with M. tuberculosis in the presence of a higher mycobacterial burden (6, 7).

Another important pro-inflammatory cytokine is TNF; Considering that TNF also significantly contributes to host defense in pulmonary mycobacterial infection (11), it is possible that IL-1 and TNF act together in protecting the host against mycobacterial infection. We previously established such an interaction between IL-1 and TNF during pneumococcal pneumonia (12). Future studies are warranted to establish the role of TNF in host defense against this virulent strain of M. kansasii.

In HIV infected patients not only the cellular immune responses are disturbed but the innate immune system is affected as well. For example, peripheral blood neutrophils, monocytes and alveolar macrophages from patients with HIV infection have been reported to be defective in their capacity to produce leukotrienes that are important proinflammatory mediators in the lung (13). Moreover, co-infection with non-HIV pathogens and associated TLR triggering have been postulated to be important exogenous factors that influence the severity and rate of disease progression in HIV+ individuals (14). Natural Killer (NK) cells and NK T cells are depleted and the number and function of circulating dendritic cells have been inversely correlated to viremia (15). Several of these processes affect the local inflammatory response in which IL-1 plays an important role.

In conclusion, our findings strengthen the notion that local innate host response and innate clearance mechanisms are sufficient to eliminate pulmonary infection with respiratory tract infection by this emerging pathogen.

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References


