Host-pathogen interactions during (myco)bacterial respiratory tract infections
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Citation for published version (APA):
CD4$^+$ cells play a limited role in murine lung infection with *Mycobacterium kansasii*
Abstract

*Mycobacterium* (*M.*) *kansasii* has emerged as an important nontuberculous mycobacterium that can cause severe infection in the immuno-compromised host, especially in HIV infected patients. However, little is known about the pathogenesis of this infection. Since patients suffering from *M. kansasii* infection are severely compromised in their cellular immune response, we studied the course of infection in mice knock-out (KO) for CD4+ cells. Wild-type (WT) mice and CD4+ KO mice were infected with $10^5$ CFU of *M. kansasii*. Although previously shown to be susceptible to *M. tuberculosis* infection, CD4+ KO mice demonstrated no impairment in clearing infection with *M. kansasii* when compared to WT animals despite reduced pulmonary inflammation (reduced granuloma formation and lymphocyte infiltration in the lungs). Pulmonary IFNγ levels and *M. kansasii*-induced IFNγ production by splenocytes from infected animals were reduced in CD4+ KO mice, confirming that these mice were defective in the *M. kansasii* specific Th1 immune response. Furthermore, mice deficient for IFNγ Interleukin (IL)-12p35, IL-12p40 or IL-18 also displayed a normal host defense against pulmonary infection with *M. kansasii*. These data suggest that CD4+ cells, IFNγ and an intact Th1 response play a limited role in protective immunity against pulmonary *M. kansasii* infection.
Introduction

*Mycobacterium* (*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 human immunodeficiency virus (HIV) infection or in patients with pre-existing pulmonary disease like asthma and chronic obstructive pulmonary disease (1-4). Since the start of the AIDS epidemic, a vast increase in *M. kansasii* infection incidence has been observed (5). However, little is known about pathogenicity, mode of transmission and natural reservoir of *M. kansasii*. The organism has been recovered occasionally from rivers and lakes but also from tap water, showerheads, and drinking water distribution systems and is thought to be acquired from the environment rather than from contact with infected patients. Unlike *M. tuberculosis*, culturing of *M. kansasii* from human sources is not exclusive proof of disease: as many as one-third of isolates has been reported to represent colonization or indolent infection of the respiratory tract rather than disease (6).

During pulmonary infection by *M. tuberculosis* or other pathogenic nontuberculous mycobacteria like *M. avium* complex, an appropriate T helper (Th) 1 response is of utmost importance to restrain the infection (7, 8). Mice lacking CD4+ T cells, IFNγ or IL-12p40 are highly susceptible to these mycobacterial infections (9-16). Because the Th1 response is severely impaired in patients with HIV infection and because AIDS patients are the main group suffering from *M. kansasii* infection, we were interested to determine whether the host response to *M. kansasii* is indeed dependent on a functional Th1 response. We therefore established a murine model of *M. kansasii* pulmonary infection and subsequently compared the immune response in CD4+ knockout (KO) and normal wild type (WT) mice. In contrast to pulmonary infection with *M. tuberculosis* or nontuberculous mycobacteria other than *M. kansasii*, the deficiency of CD4+ cells did not render the mice more susceptible to *M. kansasii*. In line, mice lacking Th1 mediators like IFNγ, IL-12p35 or p40 were not different in their host defense response from WT mice. These data lead us to hypothesize that *M. kansasii* infection can only develop in a complex form of immunodeficiency involving not only single mediators and/or cell types but several different parts of the innate and adaptive immune system. For example, HIV infected patients have been found to have reduced numbers and function of macrophages, dendritic cells, natural killer (NK) cells, NK T cells and a diminished production capacity of pro-inflammatory mediators like chemokines and leukotrienes (17-22).
Material and Methods

Mice
Six- to 8-week-old age and sex-matched CD4⁺, IFNγ, IL-12p35 and IL-12p40 KO mice on a C57BL/6 background and WT C57Bl/6 control mice were purchased from Jackson Laboratory (Bar Harbor, Maine). IL-18 KO mice, back-crossed 6 times to C57BL/6 background, were generated as described previously (23); age and sex-matched C57BL/6 WT mice were used as their controls (Harlan Spague Dawley, The Hague, The Netherlands). The Animal Care and Use Committee of the University of Amsterdam approved all experiments.

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-80. 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. Lung infection was induced as described previously (24-26). Briefly, mice were anesthetized by inhalation with isoflurane (Abbott Laboratories LTD., Kent, United Kingdom) and infected intranasally (i.n.) with M. kansasii in 50 μl saline. At the time-points indicated in the results section, 5-8 mice per group were killed, lungs were removed aseptically, and homogenized in 5 volumes of sterile 0.9 % NaCl. Ten-fold dilutions were 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. IFNγ, IL-4, TNF, IL-1β, KC and MIP-2 were measured using specific enzyme-linked immunosorbent assays (ELISA’s, R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. For detection of IL-6 a Bio-Plex Cytokine Array was used (27) (Bio-Rad Laboratories, Inc., Hercules, CA). The detection limits were 62 pg/ml for IL-4, TNF, IL-1β and MIP-2, 32 pg/mL for IFNγ, 37 pg/ml for KC and 7.8 pg/ml for IL-6.

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 (24, 25).
Erythrocytes were lysed with ice-cold isotonic NH$_4$Cl solution (155 mM NH$_4$Cl, 10 mM KHCO$_3$, 0.1 mM EDTA, pH 7.4), the remaining cells were washed twice with RPMI 1640 (Bio Whittaker, Verviers, Belgium), and counted by using a hemocytometer. The percentages of macrophages, PMNs and lymphocytes were determined using cytopsin preparations stained with hematoxilin and eosin.

Flow cytometric analysis

Lung cell suspensions obtained from infected mice were analyzed by flow cytometry using FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA) as described previously (24, 25). Cells were brought to a concentration of $4 \times 10^6$ cells per mL of FACS buffer (PBS supplemented with 0.5 % BSA, 0.01% NaN$_3$ and 0.35 mM EDTA). Immunostaining for cell surface molecules was performed for 30 minutes at 4 °C using directly labeled antibodies (abs) against CD3 (CD3-phycoerythrin), CD4 (CD4-CyChrome), CD8 (CD8-FITC and CD8-peridinin chlorophyll protein) and CD69 (CD69-FITC). All abs were used in concentrations recommended by the manufacturer (Pharmingen, San Diego, CA). After staining, cells were fixed in 2 % paraformaldehyde, and T cell surface molecules were analyzed on CD3$^+$ cells within the lymphocyte gate.

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.

Splenocyte stimulation

Single cell suspensions were obtained by crushing spleens through a 40-μm cell strainer (Becton Dickinson, Franklin Lakes, NJ) as described (24, 25). Erythrocytes were lysed with ice-cold isotonic NH$_4$Cl solution (155 mM NH$_4$Cl, 10 mM KHCO$_3$, 0.1 mM EDTA, pH 7.4); the remaining cells were washed twice with RPMI 1640 (Bio Whittaker, Verviers, Belgium) supplemented with 10 % FCS and 1 % antibiotic-antimycotic (GiboBRL, Life Technologies, Rockville, MD). Cells were seeded in 96-well round bottom culture plates at a cell density of $1 \times 10^6$ cells per well in quadruplicate, and stimulated with $2 \times 10^5$ heat-killed (HK) M. kansasii (heat killing: 20 min in 80 °C waterbath). As controls, splenocytes from uninfected WT mice and
M. tuberculosis infected animals (i.n. inoculation with $10^5$ CFU of M. tuberculosis H37Rv 5 weeks earlier) were stimulated with $2 \times 10^5$ HK M. kansasii or purified protein derivative (PPD; Statens Seruminstitut, Copenhagen, Denmark) respectively. Supernatants were harvested after 48-h incubation at 37 °C in 5 % CO₂, filter-sterilized and stored at -20 °C until ELISA was performed.

Statistical analysis

All values are expressed as mean ± SEM. Comparisons were done with Mann-Whitney U tests using GraphPad Prism version 4.00, GraphPad Software (San Diego, CA). When comparing two groups at multiple time points two way analysis of variance was used. Values of $P<0.05$ were considered statistically significant.

Results

Host defense against M. kansasii infection does not rely on CD4⁺ cells

Host defense against mycobacterial infection, in particular tuberculosis, is strongly dependent on the presence of CD4⁺ T cells and IFNγ, the main type 1 cytokine produced by this cell type (7, 8). To determine the role of CD4⁺ T cells in the immune response to pulmonary infection with M. kansasii, we infected CD4⁺ KO and normal C57BL/6 WT mice with this organism. Prior to these experiments, we first established that C57BL/6 WT mice displayed progressively declining mycobacterial loads in their lungs during a 20-week follow-up period after i.n. infection with M. kansasii at doses up to $10^6$ CFU; the infection rarely disseminated to distant organs: liver and spleen cultures were positive for mycobacteria only during the first weeks of infection and bacterial counts were low (data not shown). For subsequent experiments with CD4⁺ KO mice we chose an infectious dose of $10^5$ CFU, anticipating that whereas this dose would be effectively cleared by WT mice, this would not be the case in KO mice with a defective Th1 response. Much to our surprise, CD4⁺ KO mice demonstrated decreasing mycobacterial loads during an 8
week follow-up period after i.n. infection with *M. kansasii* comparable to that observed in WT mice (fig. 1).

![Graphs showing IFNγ and IL-4 levels in WT and CD4 KO mice.](image)

**Table 1: Inflammation scores.** Groups of 8 wild-type (WT) C57BL/6 and CD4 KO knock-out (KO) mice were i.n. inoculated with $10^5$ *M. kansasii*. After 4 and 8 weeks, inflammation was scored from H&E stained slides (for details, see Methods). Data are expressed as mean ± SEM. Differences between groups were calculated by Mann-Whitney U test. *P<0.01 versus WT mice.

<table>
<thead>
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<th>Week</th>
<th>WT</th>
<th>CD4 KO</th>
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<tr>
<td>4</td>
<td>7.1 ± 0.6</td>
<td>3.7 ± 0.5*</td>
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<tr>
<td>8</td>
<td>8.4 ± 1.1</td>
<td>7.1 ± 0.8</td>
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**Figure 2:** CD4 KO mice have reduced IFNγ lung levels. Pulmonary levels of IFNγ (A, B) and IL-4 (C, D) after 4 (A, C) and 8 (B, D) weeks of infection with $10^5$ *M. kansasii* CFU. IFNγ and IL-4 were measured in lung homogenates. Data are means ± SEM of 8 mice per group at each time-point. *P<0.05 versus WT mice.

**CD4 KO mice display reduced pulmonary IFNγ levels**

Like *M. tuberculosis*, *M. kansasii* is an intracellular pathogen residing in macrophages (28, 29). Therefore pulmonary macrophages presumably play an important role in clearing this mycobacterium. IFNγ is a typical Th1 cytokine and considered a key cytokine in host defense against pulmonary *M. tuberculosis* (7, 8). Amongst other functions, IFNγ activates macrophages to phagocytose and kill intracellular pathogens (30, 31). We therefore measured IFNγ in lung homogenates of infected WT and CD4 KO animals. At both 4 and 8 weeks post infection, pulmonary IFNγ levels were reduced in CD4 KO animals pointing to a reduced local Th1 response (fig. 2). To gain more insight in the Th2 response in WT and CD4 KO mice, we measured IL-4 in lung homogenates. At both time-points, no differences in IL-4 levels were detected locally (fig. 2).

**Reduced pulmonary inflammation in CD4 KO mice**

During *M. tuberculosis* infection, the formation of granulomas is of utmost importance to contain the infection. In mice, granulomas are formed by foamy macrophages and CD4+ and CD8+ lymphocytes (8). To obtain insight into the role of CD4+ cells in the generation of a pulmonary inflammatory response during *M. kansasii* infection, we compared cell recruitment and the cellular composition of
Table 2: Total and differential cell counts. Total leukocyte counts, differential cell counts (x10^3/mL) and T lymphocytes subset counts in lungs of WT and CD4⁺ knock-out (KO) mice 4 weeks after i.n. infection with 10⁵ CFU of M. kansasii. Data are mean ± SEM of 8 mice per group. Differences between groups were calculated by Mann-Whitney U test. *P<0.05 versus WT mice.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Møs</th>
<th>PMNs</th>
<th>Lymphocytes</th>
<th>CD8⁺</th>
<th>CD8⁺/CD69⁺</th>
<th>CD4⁺</th>
<th>CD4⁺/CD69⁺</th>
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<td>x 10^3/mL</td>
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<tr>
<td>WT</td>
<td>623 ± 103</td>
<td>311 ± 52</td>
<td>194 ± 32</td>
<td>117 ± 22</td>
<td>34 ± 7</td>
<td>3 ± 1</td>
<td>77 ± 15</td>
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<tr>
<td>CD4⁺ KO</td>
<td>477 ± 49</td>
<td>251 ± 30</td>
<td>172 ± 20</td>
<td>53 ± 92*</td>
<td>40 ± 7</td>
<td>3 ± 1</td>
<td>ND</td>
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</table>

The immune response to M. kansasii infection is not dependent on IFNγ, IL-12 or IL-18

The production of IFNγ is stimulated by IL-12, in particular in the presence of IL-18 (32). In line with the important role for IFNγ in host defense against M. tuberculosis not only IFNγ KO mice, but also p35 KO, p40 KO and IL-18 KO mice all demonstrated a reduced resistance against infection with this mycobacterium (11-13, 33-35). To obtain further proof for our finding that the Th1 response and thus IFNγ do not contribute to host defense against M. kansasii infection, we infected IFNγ KO, p35
KO, p40 KO and IL-18 KO mice with $10^5$ CFU of this microorganism and determined mycobacterial loads in their lungs 4 weeks thereafter. In line, no differences were found between the number of *M. kansasii* CFU in lungs of these KO mice and lungs of WT mice (fig. 4). In a second experiment, we infected WT and IFNγ KO mice with $10^5$ CFU of *M. kansasii* and followed them for 12 weeks. No differences in bacterial loads were detected between WT and IFNγ KO mice during this observation period.

![Figure 3](image.png)

**Figure 3:** Reduced granuloma formation and lymphocyte influx in lungs of CD4⁺ KO mice early in infection. Representative slides of lungs of WT (A, C) and CD4⁺ KO mice (B, D) infected with $10^5$ *M. kansasii* CFU 4 (A, B) and 8 (C, D) weeks earlier. After 4 weeks of infection, lungs of CD4⁺ KO showed reduced inflammation and granuloma formation. Eight weeks after infection, no difference in histopathology was observed. Hematoxilin and eosin staining; original magnification x 10.

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>CD4⁺ KO</th>
<th>WT</th>
<th>CD4⁺ KO</th>
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<tbody>
<tr>
<td>TNF</td>
<td>88 ± 3</td>
<td>83 ± 2</td>
<td>125 ± 18</td>
<td>63 ± 4*</td>
</tr>
<tr>
<td>IL-1β</td>
<td>400 ± 38</td>
<td>361 ± 29</td>
<td>378 ± 47</td>
<td>244 ± 15*</td>
</tr>
<tr>
<td>IL-6</td>
<td>247 ± 5</td>
<td>221 ± 9</td>
<td>212 ± 19</td>
<td>220 ± 10</td>
</tr>
<tr>
<td>KC</td>
<td>149 ± 9</td>
<td>107 ± 10</td>
<td>178 ± 13</td>
<td>127 ± 18*</td>
</tr>
<tr>
<td>MIP-2</td>
<td>119 ± 18</td>
<td>130 ± 18</td>
<td>118 ± 17</td>
<td>84 ± 15*</td>
</tr>
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</table>

**Table 3:** Cytokine and chemokine profile during *M. kansasii* infection. WT and CD4⁺ KO mice were i.n. inoculated $10^5$ *M. kansasii*. After 4 and 8 weeks, mice were sacrificed and levels of TNF, IL-1β, IL-6, KC and MIP-2 (all in pg/mL) were determined in lung homogenates. Data are mean ± SEM of 8 mice per group. Differences between groups were calculated by Mann-Whitney *U* test. *P<0.05 versus WT mice.
Figure 4: Deficiency of IFNγ, IL-12p40, IL-12p35 or IL-18 does not influence mycobacterial load in lungs. Mycobacterial loads in lungs: WT (closed symbols) and IFNγ KO (A), IL-12p35 KO (B), IL-12p40 KO (C) and IL-18 KO (D) (KO; all open symbols) were infected i.n. with 10^5 M. kansasii CFU. After 4 weeks of infection, mice were sacrificed and bacterial loads were determined in lung homogenates. Data are means ± SEM of 6 to 8 mice per group.

(FIG. 5). Of note, the presence of p35, p40 and IL-18 was of strong importance for an intact antigen-specific IFNγ production by infected splenocytes, i.e. splenocytes harvested from p35 KO, p40 KO and IL-18 KO mice 4 weeks postinfection released significantly less IFNγ upon stimulation with heat-killed M. kansasii than splenocytes obtained from WT mice (fig. 6). This difference in IFNγ production was comparable to the reduced production of IFNγ in CD4^+ KO splenocytes upon stimulation with heat-killed M. kansasii (fig. 6A). Compared to PPD induced IFNγ production by PPD stimulated splenocytes obtained from M. tuberculosis infected

Figure 5: IFNγ is not important for the long-term clearance of M. kansasii from the lungs. Mycobacterial loads in lungs: WT (closed symbols) and IFNγ KO (open symbols) were i.n. infected with 10^5 M. kansasii CFU. After 1 day, 1, 2, 4, 6, 9 and 12 weeks of infection, mice were sacrificed and bacterial loads were determined in lung homogenates. Data are means ± SEM of 3 (1 day) or 5 mice per group at each time point. For comparison of groups two way analysis of variance on log transformed data was used and was not found to be significant.
Figure 6: Reduced IFNγ response to *M. kansasii* specific antigens by splenocytes. Splenocytes were isolated from infected WT (filled bars) and CD4+ KO (A), IFNγ KO (B), IL-12p35 KO (C), IL-12p40 KO (D) and IL-18 KO (E) mice and stimulated with HK *M. kansasii* for 48 h. Control stimulations: stimulation with HK *M. kansasii* of splenocytes from uninfected mice (F, right bar) and stimulation with PPD of splenocytes from WT mice i.n. infected with *M. tuberculosis* 5 weeks earlier (F, left bar). IFNγ was measured in supernatants and expressed in pg/mL. Data are mean ± SEM of 4 to 8 mice per group. **P<0.01 and ***P<0.001 versus WT

WT mice, IFNγ levels were low in culture supernatants of HK *M. kansasii* stimulated splenocytes from *M. kansasii* infected animals (fig. 6F). Nevertheless, in all cases the amount IFNγ produced by splenocytes from infected WT animals was significantly increased compared to the IFNγ produced by spleen cells from uninfected mice (fig. 6F). To study the local IFNγ response, we also measured IFNγ in lung homogenates obtained from infected KO and WT mice. In contrast to IFNγ production by splenocytes, pulmonary IFNγ concentrations did not differ between WT and p35, p40 and IL-18 KO mice, whereas as expected no IFNγ was detected in lungs of IFNγ KO animals (data not shown). Histopathology, the composition of the cellular infiltrates (determined by FACS) and pulmonary cytokine and chemokine levels did not differ between WT and IFNγ KO, p35 KO, p40 KO and IL-18 KO mice (data not shown).

**Discussion**

Although *M. kansasii* is an emerging pathogen in immunocompromised patients, little is known about host defense mechanisms against this organism. Previous models of murine *M. kansasii* infection include the susceptible beige mouse strain or thymectomized C57BL/6 mice and applied intravenous or subcutaneous injection as the route of infection (36-41). Here we established a model of pulmonary infection with *M. kansasii*. After i.n.
inoculation, the bacterium was able to persist in the lung of immune competent WT C57BL/6 mice during a 20 week follow-up period with no evidence of a proliferative phase; the infection did not cause severe disease resembling the colonization observed in immune competent humans. For comparison, immune competent WT C57BL/6 mice infected with the non-pathogenic mycobacterial strain *M. smegmatis* clear the infection during the first ten days of infection (26). Moreover this particular strain of *M. kansasii* was able to induce pulmonary disease similar to tuberculosis in healthy coal mine workers indicating this strain is able to cause disease, at least in humans.

Immunocompromised and in particular HIV infected patients are susceptible to develop clinical disease after infection with *M. kansasii* (1, 3, 5, 42, 43). In order to mimic the immune-deficient state of HIV infected patients, we used mice homozygous for the targeted mutation of CD4, that have a significant block in CD4+ T-cell development; 90% of their circulating T-cells are CD8+ (44). The CD4+ KO strain has been used as a model for advanced HIV infection and severe immunocompromised states before and is a helpful tool in case long-term absence of CD4+ T cells is studied (45, 46). Although CD4+ T cells are considered to be critical helper cells in inducing adaptive immune responses against mycobacteria other than *M. kansasii* (9, 15, 16, 47), only minor differences between normal WT animals and CD4+ KO mice were detected during 8 weeks of pulmonary infection with *M. kansasii*. Our data are in contrast with a study performed by Flory et al., in which thymectomized C57BL/6 mice treated with an anti-CD4 antibody displayed higher bacterial loads in lungs and spleens after intravenous infection with *M. kansasii* persisting throughout a 12-week observation period (40). Possible explanations for this discrepancy with our data are the route of infection (intravenous versus intranasal) and differences in virulence of the *M. kansasii* strains used.

At 4 weeks post-infection, 75% of all lymphocytes present in the lungs of CD4+ KO mice were CD8+ T cells. We presume that the remaining 25% of CD3+ cells were γδT cells or MHC class II restricted CD8+/CD4+ αβ T helper cells (9, 48). It has been shown that γδT cells undergo expansion in response to mycobacterial antigens in vitro and in vivo (49, 50) and the number of γδT cells was increased during *M. bovis* BCG infection in β2-microglobulin gene deficient mice that lack functional CD8+ T cells (51). γδT cells as well as MHC class II restricted CD8+T/CD4+ αβ T helper cells, CD8+ T cells, NK cells and macrophages are capable of producing IFNγ and this IFNγ possibly compensated for the lack of CD4+ cell help allowing control of *M. kansasii* infection. In order to investigate the role of all CD3+ T and B cells in host defense against *M. kansasii*, the use of recombination activating gene (rag) KO mice is a possibility. Herein, we chose to investigate only CD4+ T cell deficient mice since
this mouse strain more closely mimics the immunodeficiency of HIV infection, a major risk factor for developing *M. kansasii* infection. IFNγ has been implicated as a pivotal mediator of host defense against intracellular pathogens (52). The main cytokine that induces the proliferation and differentiation of T cells toward IFNγ producing Th1 cells is IL-12, a heterodimeric cytokine which consists of a p35 subunit and a p40 subunit (IL-12p70) (53). IL-18 synergizes with IL-12p70 in promoting IFNγ induction in T cells and NK cells (32). Sufficient production of IFNγ is crucial in host defense against *M. tuberculosis* and, in line with the IFNγ inducing properties of IL-12 and IL-18, not only IFNγ KO mice, but also p35 KO, p40 KO and IL-18 KO mice demonstrated a reduced resistance against infection with this mycobacterium (11-13, 33-35, 54). In addition, investigations that studied the role of IFNγ and IL-12 in host defense against *M. avium* found a similarly reduced resistance in mice deficient for either one of these cytokines (14, 55, 56). Furthermore, infection with *M. bovis* BCG was detrimental for mice deficient for IFNγ, IL-18 or p35/p40 resulting in higher bacterial burdens, increased inflammation in the lungs and a reduced Th1 response (34, 54); reviewed for IFNγ KOs in (57)). These results contrast with our current findings. Indeed, much to our surprise, even the total absence of IFNγ did not lead to increased susceptibility to pulmonary *M. kansasii* infection After *M. kansasii* infection of p35, p40 and IL-18 KO mice, a similar picture as in CD4+ KO mice was obtained: decreased IFNγ production after ex vivo stimulation of splenocytes and thus a reduced Th1 response to *M. kansasii*, but no differences in bacterial clearance. IFNγ levels produced by splenocytes obtained from infected WT mice were low compared to the concentrations produced by splenocytes obtained from *M. tuberculosis* infected animals. Nevertheless, the amount IFNγ produced by infected WT animals was significantly higher than IFNγ produced by splenocytes from uninfected WT mice. Of note, in contrast to the reduced pulmonary IFNγ concentrations in CD4+ KO mice, lung IFNγ levels were unaltered in p35, p40 and IL-18 KO mice, suggesting that the local production of IFNγ during *M. kansasii* infection does not rely on IL-12 or IL-18. Altogether these data indicate that host defense against *M. kansasii* is regulated by mechanism that at least in part differ from the IFNγ dependent mechanisms that are indispensable for protective immunity against other mycobacteria. Possibly, this striking difference is related to the relatively low virulence of *M. kansasii* in the immune competent host. Our data suggest that an intact CD4+ T cell and IFNγ driven immune response is not essential for host defense against *M. kansasii* in mice. By which mechanisms then is *M. kansasii* cleared from the lungs? It is conceivable that innate immune responses mediated by Toll-like receptors, complement and antibodies evoked a response strong enough to slowly clear the pathogen from the lung. Activated resident cells like alveolar and interstitial macrophages as well as epithelial cells produce
cytokines and chemokines that attract and activate other inflammatory cells like macrophages, monocytes, neutrophils and T cells. Possibly, these cells were sufficiently activated to phagocytose and kill \textit{M. kansasii} bacilli in the absence of a strong local and systemic IFN\textsubscript{\gamma} driven adaptive immune response. This explains why the different strains of KO mice used in this study were not more vulnerable to \textit{M. kansasii} infection, although being susceptible to \textit{M. tuberculosis} infection. In this respect it is interesting to note that we recently demonstrated that Toll-like receptor 2 is important for the effective clearance of the non-pathogenic \textit{M. smegmatis} from mouse lungs (26). Furthermore, we recently found that absence of IL-1 activity in IL-1 receptor type I KO mice lead to significantly reduced clearance of \textit{M. kansasii} from the mouse lung accompanied by an enhanced pulmonary inflammatory response (C.W. Wieland et al, manuscript in preparation). These novel findings further strengthen the notion that local innate host response and innate clearance mechanisms are sufficient to eliminate pulmonary infection with this virulent strain of \textit{M. kansasii}.

Herein, we use CD4$^+$ T cell KO mice as a model for HIV infection. Although CD4 depletion is a major characteristic of HIV pathogenesis, not only the adaptive immune response is disturbed. In HIV infected patients 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 (18). Moreover, 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 (17). 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 (58).

Our study reveals that the protective immune response against pulmonary infection with \textit{M. kansasii} is not dependent on the presence of CD4$^+$ T cells or production of IFN\textsubscript{\gamma}. These data lead us to hypothesize that \textit{M. kansasii} infection can only develop in a complex form of immunodeficiency that can not be copied by using KO mice that lack specific parts of the cellular immune system.

\textit{Acknowledgments}

We thank Ingvild Kop and Joost Daalhuisen for expert technical assistance.

\textit{References}


