Host-pathogen interactions during (myco)bacterial respiratory tract infections
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CD14 contributes to pulmonary inflammation and mortality during murine tuberculosis

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Sandrine Florquin
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Abstract

Toll-like receptors play an essential role in the innate recognition of microorganisms by the host. CD14 is one of the extracellular adaptor proteins required for recognition of Gram-negative bacteria and possibly also *M. tuberculosis*. Therefore, we intranasally (i.n.) infected wild-type (WT) and CD14 knock-out (KO) mice with a virulent strain of *M. tuberculosis*. During a 32 week observation period after *M. tuberculosis* infection, none of the CD14 KO mice succumbed whereas 57% of WT mice succumbed. When we studied bacterial growth, we found that although there was no difference in bacterial load in the main target organ lung, there was reduced growth in the liver at 5 weeks after infection. At this time point of infection, pulmonary inflammation was significantly reduced in CD14 KO mice compared to WT animals. Fewer lung leukocytes were counted in CD14 KO mice and pulmonary inflammation was reduced. These data suggest that CD14 KO mice are protected from pulmonary infection with *M. tuberculosis* due to reduction of the inflammatory response.
Introduction

Tuberculosis is a devastating disease responsible for two million deaths each year. Eight million new cases of tuberculosis occur yearly (1); in total, one-third of the world population is infected (2). Healthy individuals infected with the causative organism Mycobacterium (M.) tuberculosis develop a robust T helper 1 (Th 1) driven immune response that prevents the development of disease but is not strong enough to eradicate the bacterium (2). Microorganisms express conserved pathogen associated molecular patterns (PAMPs) that are recognized by pattern recognition receptors present on host cells. One of the most prominent families of pattern recognition receptors is the Toll-Like Receptor (TLR) family. The interaction between PAMPs, TLRs and TLR associated molecules initiates an inflammatory response enabling the host to combat the pathogen. A TLR associated molecule that contributes to TLR induced cell activation is CD14 (3). CD14 is a glycosylphosphatidylinositol anchored surface molecule present on granulocytes, monocytes and macrophages (4). Two forms of CD14 exist: membrane bound CD14 (mCD14) and soluble CD14 (sCD14). CD14 does not have an intracellular signalling domain and requires interaction with a TLR for cell activation. The best studied example of such an interaction is the binding of lipopolysaccharide (LPS) from Gram-negative bacteria by CD14 and the transfer of LPS from CD14 to TLR4. In addition to LPS, CD14 in vitro binds non-mannose-capped lipoarabinomannan (AraLAM), a prominent mycobacterial cell wall glycolipid present in fast-growing, non-pathogenic mycobacteria (5, 6). Although mannose-capped LAM (ManLAM) present in the cell wall of M. tuberculosis induces almost no effects in cell lines transfected with CD14 and/or TLRs, several effects of ManLAM can be inhibited by antibodies against CD14, suggesting that CD14 may play a role in at least some ManLAM-induced responses (7-10). Moreover, CD14 may act as an uptake receptor for mycobacteria in some cell types (11, 12) and the M. tuberculosis chaperonin 60.1 partially activates cells via a CD14 dependent mechanism (13, 14). Hence, evidence points to a role for CD14 in the interaction between mycobacteria and host immune cells in vitro.

Knowledge of the in vivo role of CD14 during mycobacterial infection is relatively limited. We reported earlier that CD14 is not important for AraLAM induced pulmonary inflammation or the antibacterial host response against avirulent M. smegmatis infection (15). In addition, although the absence of CD14 resulted in a reduced and delayed release of tumor necrosis factor-a (TNF) by macrophages infected with M. avium in vitro, CD14 knockout (KO) mice displayed an unaltered antibacterial defense and granulomatous reaction after intravenous infection with M. avium in vivo (16). Thusfar, only one study examined the contribution of CD14 to host defense against lung tuberculosis reporting that CD14 KO mice had an immune response that was indistinguishable from that in normal wild-type (WT)
mice during a 14-week follow up after aerosol *M. tuberculosis* infection (17). In the present study we sought to determine the long-term consequences of CD14 deficiency on the host response to pulmonary infection with *M. tuberculosis*. We report that CD14 KO mice are protected from mortality due to lung tuberculosis occurring in WT mice from 20 weeks after infection, which is accompanied by reduced lung inflammation in the presence of an unaltered mycobacterial load.

**Materials and Methods**

**Mice**
Pathogen-free 8 to 10 week old WT C57BL/6 mice were purchased from Harlan Sprague Dawley Inc. (Horst, The Netherlands). CD14 KO mice, backcrossed 6 times to C57BL/6 background, were obtained from the Jackson Laboratories (Bar Harbor, ME, (18)). The Animal Care and Use of Committee of the University of Amsterdam approved all experiments.

**Experimental infection**
A virulent laboratory strain of *M. tuberculosis* H37Rv (American Type Culture Collection, Rockville, MA) was grown for 4 days 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. Tuberculosis was induced as described previously (19-21). Briefly, mice were anesthetized by inhalation with isoflurane (Abott Laboratories LTD., Kent, United Kingdom) and infected intranasally (i.n.) with $10^5$ live *M. tuberculosis* H37Rv bacilli in 50 μl saline, as determined by viable counts on Middlebrook 7H11 plates. Groups of eight mice per time-point were sacrificed 2 and 5 after infection; in addition, mice that were still alive 32 weeks after infection were killed and their organs processed as described further. Lungs, liver and spleen (only 32 weeks) 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 21 days incubation at 37 °C. Numbers of CFU are provided per g of lungs.

**Histology**
Lungs were removed 2, 5 or 32 weeks after inoculation with *M. tuberculosis*, fixed in 10 % buffered formaline for 24 h, and embedded in paraffin. Hematoxilin and eosin stained slides were coded and scored from 0 (absent) to 4 (severe) for the following parameters: Interstitial inflammation, endothelialitis, bronchitis, oedema, granuloma formation and pleuritis by a pathologist blinded for groups. The total "lung inflammation score" was expressed as the sum of the scores for each parameter, the maximum being 24 (15). Confluent (diffuse) inflammatory
infiltrate was quantified separately and expressed as percentage of the lung surface.

**Flow cytometry**

Lung cell suspensions were obtained by crushing lungs through a 40-μm cell strainer (Becton Dickinson, Franklin Lakes, NJ) as described previously (19-21). Erythrocytes were lysed with ice-cold isotonic NH₄Cl solution (155 mM NH₄Cl, 10 mM KHCO₃, 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, polymorphonuclear cells (PMNs) and lymphocytes were determined using flow cytometric analysis using FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA). In addition, cells were brought to a concentration of 1 x 10⁷ cells per mL of FACS buffer (PBS supplemented with 0.5 % BSA, 0.01% NaN₃ 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-APC), CD8 (CD8-peridinin chlorophyll protein), CD69 (CD69-FITC) or GR-1 (GR-1 FITC). All abs were used in concentrations recommended by the manufacturer (BD Pharmingen, San Diego, CA). After staining, cells were fixed in 2 % paraformaldehyde, and T cell surface molecules were on CD3+ cells within the lymphocyte gate.

**Cytokine measurements**

For cytokine measurements, organ 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, and supernatants were sterilized using a 0.22 μm filter (Corning Incorporated, Corning, NY) and stored at -20 °C until assays were performed. Interferon (IFN)-γ, interleukin (IL)-4, TNF, IL-10, IL-6, KC, MIP-2 and MCP-1 were measured by ELISA using matched antibody pairs using high binding ELISA plates (Greiner Bio-One, Frieckenhausen, Germany) according to the manufacturer’s instructions (R&D Systems Inc., Minneapolis, Minnesota, USA but BD Pharmingen for MCP-1). Detection limits were 63 pg/mL for IFNγ, IL-4, TNF, IL-10, IL-6. and MIP-2, 300 pg/mL for MCP-1 and 15 pg/mL for KC.

**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). Survival curves were compared by log rank test. When comparing two groups at multiple time points two way ANOVA was used. If appropriate, ANOVA’s were
followed by Bonferroni post test. Statistical analyses of bacterial counts were performed after log transformation. Values of P<0.05 were considered statistically significant.

Results

CD14 KO mice are protected from lethality after M. tuberculosis infection
To investigate the role of CD14 in the outcome of tuberculosis, mice were inoculated with M. tuberculosis and followed for 32 weeks (fig. 1). During the first 20 weeks none of the mice in either group died. Thereafter mortality occurred among WT mice only; at the end of the 32-week observation period 9 out of 14 WT (64%) but none of the CD14 KO had died (P<0.01).

![Figure 1: CD14 mice are protected against lethality. WT (n = 14; closed symbols) and CD14 KO mice (n = 14; open symbols) were i.n. infected with 10^5 CFU of M. tuberculosis and followed for 32 weeks. P< 0.01 for differences between groups.](image)

CD14 does not influence mycobacterial outgrowth
Having established that the presence of CD14 contributes to lethality in our model of lung tuberculosis, we determined whether differences could be detected between CD14 KO and WT mice with regard to mycobacterial counts in the lungs in the early phase of infection. However, at 2 and 5 weeks after infection the numbers of M. tuberculosis CFU recovered from the lungs of WT and CD14 KO mice did not differ (fig. 2A). Moreover, lungs of mice that survived 32 weeks of infection (5 WT and 14 CD14 KO mice) contained a similar amount of bacteria (fig. 2B). Hence, these data argue against an important role for CD14 in limiting the growth of M. tuberculosis in the pulmonary compartment in both the early and late phase of the infection.

CD14 KO mice display less lung inflammation
To obtain insight into the possible mechanism by which CD14 contributes to lethality, we performed histopathologic analyses of lung tissue slides prepared
CD14 in tuberculosis

<table>
<thead>
<tr>
<th>Group</th>
<th>2 wks postinfection</th>
<th>5 wks postinfection</th>
<th>32 wks postinfection</th>
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<tbody>
<tr>
<td></td>
<td>Lung weight (g)</td>
<td>Inflamed area (%)</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>0.14 ± 0.01</td>
<td>42 ± 4</td>
<td></td>
</tr>
<tr>
<td>CD14 KO</td>
<td>0.14 ± 0.01</td>
<td>30 ± 6</td>
<td></td>
</tr>
<tr>
<td>5 wks postinfection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>0.25 ± 0.01</td>
<td>51 ± 4</td>
<td></td>
</tr>
<tr>
<td>CD14 KO</td>
<td>0.16 ± 0.01**</td>
<td>33 ± 3**</td>
<td></td>
</tr>
<tr>
<td>32 wks postinfection</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>0.27 ± 0.02</td>
<td>80 ± 4</td>
<td></td>
</tr>
<tr>
<td>CD14 KO</td>
<td>0.27 ± 0.03</td>
<td>62 ± 1**</td>
<td></td>
</tr>
</tbody>
</table>

Table I: Lung inflammation. Groups of 8 WT C57BL/6 and CD14 KO mice were i.n. inoculated with 10^5 M. tuberculosis. After 2 and 5 weeks, right lung weights were measured and % inflamed lung parenchyma was scored from H&E stained slides of left lungs (For details, see Methods). Data obtained at 32 weeks are derived from 5/14 WT and 14/14 CD14 KO mice that survived the 32-week observation period shown in figure 1. Data are expressed as mean ± SEM. *P<0.05 and **P<0.01; versus WT control.

from CD14 KO and WT mice 2, 5 or 32 weeks after infection. At 2 weeks, both groups displayed granulomatous inflammation, generally located around small bronchi and vessels (fig. 3A and 3B). Not only the architecture, but also the extent and severity of the pulmonary inflammatory response were similar in CD14 KO and WT mice at this time point (table I). At 5 weeks after infection, however, profound differences between WT and CD14 KO mice were found. At this time point, pulmonary inflammatory infiltrate in lungs from WT mice had become denser and more diffuse throughout the lungs. In contrast, the extent and severity of the granulomatous inflammatory infiltrate from lungs of CD14 KO were comparable to those observed at 2 weeks after infection (fig. 3C and 3D). Indeed, lung weights and the percentage of inflamed lung parenchyma had increased in the lungs of WT mice (table I). Lungs of CD14 KO did not show progression of lung inflammation from 2 to 5 weeks after infection; at 5 weeks lung weights and the percentage of lung parenchyma involved were lower in CD14 KO than in WT mice (both P < 0.01, table I). Moreover, pleuritis was increased in lungs of WT mice. Together this resulted in lower lung inflammation scores in CD14 KO mice compared to WT mice (9.5 ± 0.7 versus 13.9±0.4 respectively, P < 0.01).

Figure 2: Unaltered pulmonary bacterial growth in CD14 KO mice. Wild-type (WT) and CD14 knock-out mice (KO) were infected i.n. with 10^5 CFU of M. tuberculosis. Two and 5 weeks after infection, mice were sacrificed and bacterial loads were determined in homogenates of lung and liver. Data are presented as means ± SEM of 7-8 mice per group per time point.
Figure 3: Histopathology. Representative lung histology of wild-type (WT; A and C) and CD14 knock-out (KO; B and D) mice 2 (A, B) and 5 (C, D) weeks after i.n. infection with 10^5 CFU of M. tuberculosis. The lung sections are representative for 7-8 mice per group per time point. H&E staining, magnification x 5, insets x 20.

<table>
<thead>
<tr>
<th></th>
<th>Leukocytes</th>
<th>Møs</th>
<th>PMNs</th>
<th>Lymphocytes</th>
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<tbody>
<tr>
<td></td>
<td>x 10^5/mL</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>2 wks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>19 ± 6</td>
<td>43 ± 2</td>
<td>10 ± 1</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>CD14 KO</td>
<td>12 ± 2</td>
<td>48 ± 2</td>
<td>9 ± 1</td>
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<tr>
<td>WT</td>
<td>62 ± 1</td>
<td>36 ± 2</td>
<td>4 ± 1</td>
<td>52 ± 2</td>
</tr>
<tr>
<td>CD14 KO</td>
<td>28 ± 1**</td>
<td>38 ± 2</td>
<td>2 ± 1</td>
<td>51 ± 3</td>
</tr>
</tbody>
</table>

Table II: Effect of CD14 deficiency on total and differential lung cell counts. *Total leukocyte counts (x10^5/mL) and differential cell counts counts in lungs of wild-type (WT) and CD14 knock-out (KO) mice 2 and 5 weeks after i.n. infection with 10^5 CFU of M. tuberculosis. **P<0.01 versus WT.

To obtain more insight into the role of CD14 in the lung inflammatory reaction to tuberculosis, we analyzed lung tissue slides harvested from the mice that were still alive after the 32-week study described above. Although 9/14 WT animals had already died, causing a bias since the animals with the most severe lung damage are expected to die earlier, CD14 KO mice still showed less lung inflammation as reflected by both the extent and severity of the inflammatory reaction and the percentage of inflamed lung tissue mice (both P < 0.01, table I). Hence, these data
suggest that CD14 KO mice develop less pulmonary inflammation during experimental tuberculosis.

**CD14 KO mice display a reduced cell recruitment to the lungs**

To obtain more insight into the cellular composition of the pulmonary infiltrates in CD14 KO and WT mice, we prepared whole lung cell suspensions at 2 and 5 weeks after infection (table II). In accordance with the histopathologic analyses, at 2 weeks after infection leukocyte counts and the percentages of macrophages, lymphocytes and neutrophils were similar in lungs of CD14 KO and WT mice. At 5 weeks after infection, however, total leukocyte counts were higher in WT mice than in lungs of CD14 KO mice (P < 0.01), whereas the percentages of macrophages, lymphocytes and neutrophils remained similar in both mouse strains. To determine whether the reduction in absolute lymphocyte numbers in the lungs of CD14 KO mice was restricted to a certain subset, we analyzed whole lung CD3+ lymphocytes with respect to expression of CD4, CD8 and the activation marker CD69 (table III). This revealed that the percentages of CD4+ and CD8+ T cells within the CD3+ population did not differ between the two mouse strains. Moreover, in both groups CD4+ and CD8+ T cells equally expressed CD69.

<table>
<thead>
<tr>
<th>Lung</th>
<th>CD4+</th>
<th>CD4+/CD69+</th>
<th>CD8+</th>
<th>CD8+/CD69+</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 wks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>65 ± 3</td>
<td>24 ± 2</td>
<td>25 ± 2</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>CD14 KO</td>
<td>67 ± 4</td>
<td>20 ± 3</td>
<td>22 ± 3</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>5 wks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>62 ± 2</td>
<td>18 ± 1</td>
<td>30 ± 2</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>CD14 KO</td>
<td>59 ± 2</td>
<td>19 ± 1</td>
<td>28 ± 1</td>
<td>11 ± 1</td>
</tr>
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</table>

**Table III**: Effect of CD14 deficiency on T cell subsets in lungs during tuberculosis. T lymphocyte subsets in lungs WT and CD14 KO mice 2 and 5 weeks after i.n. infection with 105 CFU of *M. tuberculosis*. Data are presented as the percentage positive cells in the CD3+ gate. Data are mean ± SEM of 6-8 mice.

**Cytokine and chemokine response**

Cytokines and chemokines play a pivotal role in the regulation of the immune response to tuberculosis (22, 23). Therefore we measured the concentrations of proinflammatory cytokines (IFN-γ, TNF, IL-1β, IL-6), anti-inflammatory cytokines (IL-4, IL-10) and chemokines (MIP-2, KC, MCP-1) in lung homogenates obtained 2 and 5 weeks after infection (table IV). The concentrations of most mediators were similar in lungs of CD14 KO and WT mice at each time point. At 2 weeks after infection the concentrations of the prototypic type 1 cytokine IFN-γ and the CXC chemokine MIP-2 were higher in CD14 KO (P < 0.05 versus WT mice) whereas the CC chemokine MCP-1 was reduced (P < 0.01 versus WT mice). Lung levels of the anti-inflammatory IL-10 were higher as well at 2 weeks after infection (P < 0.01 versus WT mice). At 5 weeks after infection, when pulmonary inflammation was attenua-
Table IV: Effect of CD14 deficiency on pulmonary cytokine and chemokine levels. Cytokine and chemokine levels (in ng/mL) in lung homogenates of wild-type (WT) and CD14 knock-out (KO) mice 2 and 5 weeks after i.n. infection with 10⁵ CFU of *M. tuberculosis*. Data are mean ± SEM of 7 or 8 mice per group. *P<0.05 and **P<0.01 versus WT mice.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>2 wks</th>
<th>5 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ</td>
<td>0.81 ± 0.09</td>
<td>1.50 ± 0.16*</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.06 ± 0.01</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>TNF</td>
<td>0.57 ± 0.09</td>
<td>0.73 ± 0.11</td>
</tr>
<tr>
<td>IL-1β</td>
<td>4.11 ± 1.06</td>
<td>2.87 ± 0.61</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.37 ± 0.35</td>
<td>3.39 ± 0.77*</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.26 ± 0.07</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>KC</td>
<td>0.87 ± 0.18</td>
<td>1.72 ± 0.40</td>
</tr>
<tr>
<td>MIP-2</td>
<td>0.64 ± 0.18</td>
<td>1.30 ± 0.23*</td>
</tr>
<tr>
<td>MCP-1</td>
<td>9.77 ± 0.89</td>
<td>10.37 ± 0.59</td>
</tr>
</tbody>
</table>

ted in CD14 KO mice, these mice had lower lung IL-1β concentrations and no differences in chemokine levels were found.

**Discussion**

TLRs are pivotal for the induction of the innate immune response against various micro-organisms entering the host. CD14 is a pivotal component of the LPS recognition complex and as a consequence thereof considered important for host defense against Gram-negative infections (24, 25). In addition to binding LPS, CD14 has been implicated in the *in vitro* recognition of LAM present in the mycobacterial envelope. We reported earlier that CD14 is not important for AraLAM induced pulmonary inflammation or the acute antibacterial response against avirulent *M. smegmatis* infection in vivo (15). In the present study we sought to determine the role of CD14 during chronic infection with virulent *M. tuberculosis*. We here demonstrate that CD14 KO mice are strongly protected against lethality during lung tuberculosis, which was associated with reduced pulmonary inflammation in the presence of an unaltered outgrowth of *M. tuberculosis*.

To the best of our knowledge, only one previous study examined the role of CD14 during pulmonary infection with *M. tuberculosis* (17). In that study Reiling et al. demonstrated that pulmonary mycobacterial loads were similar in CD14 KO and WT lungs during a 14-week period after aerosol infection with *M. tuberculosis*. During this period mortality did not occur in either mouse strain and no differences in granuloma formation or pulmonary levels of TNF, IL-12p40 or IFNγ were detected between CD14 KO and WT mice (17). Although the aerosol delivery can not be directly compared with our intranasal infection route, pulmonary mycobacterial loads were in the same range as in the present investigation from 3 to 14 weeks.
after infection (17). We confirmed and extended these findings by showing that mycobacterial loads remained similar in CD14 KO and WT mice up to 32 weeks after infection. Two important differences exist between the earlier study (17) and ours. First, Reiling et al. used CD14 KO on a BALBc genetic background, whereas we used CD14 KO C57BL/6 mice. Notably, BALBc mice display a susceptible Th2 skewed genetic background whereas C57BL/6 mice are relatively more resistant due to a Th1 skewed phenotype (18, 26). More importantly, we followed mice beyond the initial 14 weeks of the infection, revealing that WT mice but not CD14 KO mice started to succumb from tuberculosis from week 20 onward. In addition, Reiling et al. did not show the impact of CD14 deficiency on histopathology in detail (17); we here demonstrate that CD14 KO mice generate an attenuated inflammatory response in lung tissue, as reflected by lung weights, semi-quantitative analysis of histology slides and the numbers of leukocytes recruited to the pulmonary compartment. Together these data suggest that CD14 contributes to the development of a chronic inflammatory response in the lung during tuberculosis which apparently negatively influences the outcome of the infection. In line, we recently found enhanced *M. tuberculosis* induced mortality due to an uncontrolled inflammatory response in mice with a loss-of-function mutation in the *thrombomodulin* gene; the adverse outcome of these mice was not accompanied by increased bacterial outgrowth (21). Of note, Ehlers et al. reported similar bacterial loads in lungs, liver and spleen of CD14 KO and WT mice during an 8-week follow up after intravenous infection with *M. avium* (16).

It appears as striking that several studies investigating the role of TLRs and TLR associated molecules in mycobacterial infection are divergent in their results (15, 17, 27-37). The results of these studies differ from none, moderate or even strong effects due to the lack of a TLR or TLR associated molecule. To our knowledge, our study is novel in demonstrating that CD14 deficiency results in protection from inflammation induced destruction of the lungs. In contrast, studies in which protective roles for TLR2, TLR4 or MyD88 were found, deficiencies of these molecules resulted in exaggerated inflammatory responses most likely due to the increased mycobacterial load, providing a more potent proinflammatory stimulus (29, 31-34, 36). It remains to be established which signalling receptor(s) mediate the CD14 dependent inflammation observed in WT mice in the present study.

Interestingly, CD14 has been implicated in the recognition of apoptotic cells and bodies, resulting in induction of phagocytosis by macrophages (38, 39); macrophages from CD14 KO did not clear apoptotic cells and bodies but were still able to produce anti-inflammatory mediators associated with recognition and phagocytosis (39). Although a prolonged anti-inflammatory impulse by the persistence of apoptotic cells and bodies might influence lung inflammation, we
did not detect any differences in numbers of apoptotic bodies in our histopathological slides (data not shown).

Although some evidence suggests that CD14 is a binding and uptake receptor for *M. tuberculosis* (11, 12), our data can not be explained by such a CD14 function. Indeed, we did not detect differences in the growth of mycobacteria in lungs of CD14 KO and WT mice and *M. tuberculosis* can also bind and/or enter macrophages via several other receptors, including complement receptors, the mannose receptor, scavenger receptors, Fcγ receptor, surfactant protein A receptor and CD44 (19, 40), making an irreplaceable role for CD14 in these processes less likely. Moreover, it should be noted that the role of CD14 as a receptor for *M. tuberculosis* on human mononuclear phagocytes remains controversial (41).

CD14 KO mice not only lack cell surface CD14 but also soluble CD14. Soluble CD14 behaves as an acute phase protein and elevated levels have been found in bronchoalveolar lavage fluid and serum of patients with active tuberculosis (42-44). The function of soluble CD14 in tuberculosis is unknown and our present study does not discriminate between the roles of cell surface and soluble CD14. Nonetheless, our data suggest that CD14 is important for eliciting lung inflammation during *M. tuberculosis* infection which during progressive disease can result enhanced mortality.

**Acknowledgments**

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**References**

CD14 in tuberculosis


