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

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Mice lacking murine SIGNR1 have a normal host defense against *Mycobacterium tuberculosis*
Abstract

*Mycobacterium (M.) tuberculosis* and the associated disease tuberculosis is a main health risk causing many deaths world-wide each year. *M. tuberculosis* targets dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN). This interaction induces Interleukin (IL-10) production by the DC and prevents DC maturation, possibly leading to immunosuppression. We investigated the role of a murine homolog of DC-SIGN, SIGN Related 1 (SIGNR1), in a model of *M. tuberculosis* infection using SIGNR1 deficient (KO) mice. In contrast to the DC-specific DC-SIGN, mouse SIGNR1 is expressed by macrophage subpopulations in lymph nodes, spleen and peritoneal cavity but not by alveolar macrophages even not during infection. Although SIGNR1 is able to bind both ManLAM and *M. tuberculosis in vitro*, we did not detect any differences in mycobacterial loads of lung, spleen, liver and draining lymph nodes after 1 day, 2 and 5 weeks of infection resulting in similar survival rates. Moreover, despite increased T cell activity in SIGNR1 KO mice early during infection, pulmonary levels of IL-10 and IFNγ were similar in both groups. Therefore despite our observation of an increased IFNγ production by splenocytes in SIGNR1 KO mice early in infection suggesting an immunomodulatory role for SIGNR1 towards a Th1 response, our data suggest that the role of SIGNR1 is limited during *M. tuberculosis* infection *in vivo.*
Introduction

*Mycobacterium tuberculosis* and the associated disease tuberculosis are main threats to mankind, with one-third of the world population being infected (1). Over 8 million new cases of tuberculosis and 2 million deaths from this disease occur yearly worldwide (2). Although healthy persons infected with *M. tuberculosis* develop a solid immune response, this response is not strong enough to eradicate the bacterium. Mechanisms by which *M. tuberculosis* escapes from the host immune response have been studied in detail. Although macrophages, and not dendritic cells (DC), are the primary targets for infection by mycobacteria, DC are important for the cellular immune response. Recent data strongly suggest that C-type lectins are involved in suppressing cellular immune responses mediated by DC (3, 4).

C-type lectins recognize pathogens by binding to pathogen-specific carbohydrate residues. The human C-type lectin DC-SIGN binds to mannose-capped lipoarabinomannan (ManLAM), a major cell wall component of *M. tuberculosis* (5-7). Both membrane bound and secreted ManLAM is considered an important virulence factor of *M. tuberculosis* (8, 9). After binding to DC-SIGN, ManLAM stimulates the production of the anti-inflammatory cytokine interleukin (IL)-10 and inhibits Toll-like Receptor-induced DC maturation (5). These findings indicate that in humans, binding of ManLAM to DC-SIGN hampers DC function that might lead to suppression of the adaptive immune response against *M. tuberculosis* and possibly facilitates survival of the pathogen.

Five different homolog genes of DC SIGN were cloned in mice but the cellular expression has been elucidated only for two of these murine homologs: murine DC-SIGN (mDC-SIGN) and SIGN Related (SIGNR)-1 (10-13). mDC-SIGN is expressed by plasmacytoid pre-DC (14, 15). However, so far no ligands have been identified for mDC-SIGN. In contrast, SIGNR1 is abundantly expressed in lymph nodes by medullary and subcapsular macrophages, in spleen by marginal zone macrophages and in the liver by sinusoidal endothelial cells (13). In addition, Taylor et al. demonstrated that SIGNR1 is also expressed on resident peritoneal macrophages (12). The function and binding capacities of SIGNR1 have been studied in detail and are similar to human DC-SIGN (7, 13, 16). Recently, it was demonstrated that SIGNR1 is important in the defense against *Streptococcus* (S.) *pneumoniae* infection (17, 18). Interestingly, like DC-SIGN, SIGNR1 binds to ManLAM and *M. tuberculosis* (7, 12). Here we set out to elucidate the in vivo function of SIGNR1 in the immune defense against *M. tuberculosis*. The role of SIGNR1 in the host defense response against *M. tuberculosis* was studied using SIGNR1 deficient mice (KO) mice (17). Early during infection splenocytes from SIGNR1-deficient mice produced more IFNγ than wild-type (WT) splenic T cells, suggesting a more pronounced Th1-specific immune response. However, no differences in susceptibility to *M. tuberculosis* infection.
were observed between SIGNR-1-deficient and WT mice. Our data suggest that although SIGNR1 is able to bind ManLAM from *M. tuberculosis* in vitro, its role is limited during *M. tuberculosis* infection in vivo.

**Materials and Methods**

**Mice**
SIGNR1 KO mice were a generous gift from A. McKenzie (Cambridge, UK). C57BL/6x129 WT and SIGNR1 KO mice were bred in the animal facility of the VU University Medical Center under specific pathogen-free conditions, and were kept in the animal facilities of the VU Medical Center and the Academic Medical Center in Amsterdam, The Netherlands. Six- to 8-week-old male mice were used in all experiments and maintained in biosafety level 3 facilities. The Animal Care and Use 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 10⁵ 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 two and five weeks after infection. Lungs, liver and spleen were removed aseptically and homogenized in 5 volumes of sterile 0.9 % NaCl. 10-fold dilutions were plated on Middlebrook 7H11 agar plates to determine bacterial loads. Colonies were counted after 21 days at 37 °C. Numbers of CFU are provided per g of lungs. In order to check for infection efficacy, three mice per group were sacrificed one day post-infection. 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) stored at -20 °C until assays were performed.

**Characterization of inflammatory infiltrates in the lungs**
Lung and draining mediastinal lymph nodes cell suspensions were obtained by crushing lungs through a 40-µm cell strainer (Becton Dickinson, Franklin Lakes, NJ) as described previously (19, 20). Erythrocytes in lung cell suspensions 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 cytospin preparations stained with haematoxilin and eosin.

**Flowcytometric analysis**

Lung cell suspensions obtained from infected mice were analyzed by flowcytometry using FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA) as described previously (19, 21). 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) and CD69 (CD69-FITC). All abs were used in concentrations recommended by the manufacturer (Pharmlingen, San Diego, CA). After staining, cells were fixed in 2 % paraformaldehyde, and T cell surface molecules were analyzed within the gate containing CD3⁺ cells.

**Histology**

Lungs were removed 2 and 5 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. In addition, the percentage of inflamed lung parenchyma was scored separately. For SIGNR1 stainings the slides were deparaffinized and endogenous peroxidase activity was quenched by a solution of methanol/0.03% H₂O₂ (Merck, Darmstadt, Germany). After digestion with a 10 mmol/L sodium citrate solution, pH 6.0, non-specific binding was blocked with TENG-T (10 mM Tris, 5 mM EDTA, 0.15 M NaCl, 0.25% gelatine, 0.05% (v/v) Tween 20, pH 8) and then exposed to a goat anti-SIGNR1 antibody (R&D Systems, Minneapolis, MN). After washes, slides stained for SIGNR1 were incubated with a horseradish peroxidase-labeled rabbit anti-goat IgG2a antibody (Southern Biotech, Birmingham, AL). Slides were finally developed using 1% H₂O₂ and 3,3'-diaminobenzidine-tetra-hydrochloride (Sigma) in Tris-HCl and slides were counterstained with methyl green. The sections were mounted in glycerin gelatin. As a negative control, spleens and lungs from SIGNR1 KO mice were stained.

**Splenocyte stimulation**

Single cell suspensions were obtained by crushing spleens through a 40-µm cell
strainer (Becton Dickinson, Franklin Lakes, NJ) as described (19, 21). Erythrocytes were lysed with ice-cold isotonic NH₄Cl solution (155 mM NH₄Cl, 10 mM KHCO₃, 100 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 (Greiner Bio-One, Frieckenhausen, Germany) at a cell density of 1 x 10⁶ cells per well in quadruplicate, and stimulated with 20 μg/ml tuberculin purified protein derivative (PPD, Statens Seruminstitut, Copenhagen, Denmark). Supernatants were harvested after 48-h incubation at 37 °C in 5 % CO₂, and cytokine levels were analyzed by ELISA.

**Cytokine measurements**

Interferon (IFN)-γ, IL-4, Tumor Necrosis Factor (TNF), IL-1β, IL-10, KC and MIP-2 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). Detection limits were 63 pg/mL for IFNγ, IL-4, IL-10, TNF, IL-1β, and MIP-2 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. Statistical analyses of bacterial counts were performed after log transformation. Values of P<0.05 were considered statistically significant.

**Results**

**No differences in M. tuberculosis growth in lung, spleen or liver**

To determine the role of SIGNR1 in antibacterial defense against tuberculosis, the outgrowth of *M. tuberculosis* in lungs of SIGNR1 KO and WT mice was determined 1 day and 2 and 5 weeks after infection (fig.1). At all time points after infection, the numbers of CFU's detected in the lung were similar in SIGNR1 KO and WT animals. To study the dissemination of *M. tuberculosis*, the bacterial load in spleen and liver was measured as well. Again, no differences in bacterial growth in spleen or liver were observed between SIGNR1 KO and WT mice at the different time points (fig.1). To further assess the role of SIGNR1 in host defense against tuberculosis, 14 SIGNR1 KO and 14 WT mice were studied during a 6 months observation period after infection. A similar percentage of animals died (8 out 14 in each group) (data not shown). Thus, SIGNR1 does not influence the outgrowth or dissemination of *M.
Figure 1: Unaltered bacterial outgrowth in SIGNR1 knock-out mice. WT (closed symbols) and SIGNR1 KO mice (open symbols) were infected i.n. with $10^5$ CFU of *M. tuberculosis*. One day, 2 and 5 weeks after infection, mice were sacrificed and bacterial loads were determined in homogenates of lung, spleen and liver. Data are presented as means ± SEM of 3 (1 day) to 6-8 mice per group per time point.

Figure 2: In both WT and SIGNR1 KO a similar degree of inflammation was observed in the lung. Representative lung histology of wild-type (WT; A and C) and SIGNR1 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 6-8 mice per group per time point. H&E staining, magnification $\times 10$. 
**Table I: Effect of SIGNR1 deficiency on total and differential lung cell counts.**

<table>
<thead>
<tr>
<th></th>
<th>Cells</th>
<th>Mφs</th>
<th>PMNs</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x 10⁵/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>40 ± 6</td>
<td>42 ± 3</td>
<td>25 ± 4</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>SIGNR1 KO</td>
<td>53 ± 11</td>
<td>44 ± 5</td>
<td>28 ± 3</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>5 wks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>138 ± 57</td>
<td>37 ± 4</td>
<td>38 ± 5</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>SIGNR1 KO</td>
<td>122 ± 43</td>
<td>38 ± 7</td>
<td>34 ± 7</td>
<td>15 ± 3</td>
</tr>
</tbody>
</table>

Total leukocyte counts (x10⁵/mL) and differential cell counts counts in lungs of WT and SIGNR1 KO mice 2 and 5 weeks after i.n. infection with 10⁵ CFU of *M. tuberculosis*. Data are mean ± SEM of 6-8 mice per group per time point.

*tuberculosis* nor does it appear to be essential for survival after infection with this mycobacterium.

**No differences in histopathology in lungs of SIGNR1 KO or WT mice**

Two weeks after infection, lungs of both WT and SIGNR1 KO mice already displayed advanced tuberculosis with high scores for interstitial inflammation and inflammation of vessels and bronchi (14.4±0.6 for WT and 14.6±1.9 for SIGNR1 KO lungs; fig. 2). The diffuse infiltrate consisted mainly of mononuclear cells and no differences in the degree of inflammation could be detected between WT and SIGNR1 KO mice (fig. 2 and table I). After 5 weeks of infection lung inflammation scores increased (18.8±0.6 for WT and 17.2±1.7 for SIGNR1 KO lungs). Moreover, the percentage of lung parenchyma involved in inflammation was higher at 5 weeks post-infection and this resulted in higher total cell counts in the lungs of both groups (table I). In contrast to 2 weeks post-infection, the percentage of infiltrated PMNs increased in the lungs of both groups after 5 weeks of infection (table I). Based on these results it seems that SIGNR1 is not crucial for the inflammation of the lungs and the concomitant cellular infiltration, upon infection with *M. tuberculosis*.

**No induction of SIGNR1 in the lung during M. tuberculosis infection**

In order to study whether SIGNR1 positive cells enter the lung during tuberculosis or whether resident cells start to express SIGNR1 during infection, we stained lung slides from WT and SIGNR1 KO with a polyclonal antibody against SIGNR1. Lungs from infected WT mice did not demonstrate any positive staining at both 2 and 5 weeks after infection (fig. 3A for 2 weeks and data not shown for 5 weeks). As expected, macrophages in the marginal zones of spleens from WT mice stained positive (fig. 3B) whereas, no positive staining for SIGNR1 was visible in spleens from SIGNR1 KO mice demonstrating the specificity of the antibody used (data not shown). Thus, inflammation due to infection by *M. tuberculosis* does not induce the expression of SIGNR1 in macrophages of the lung.
SIGNR1 KO splenocytes produce increased IFNγ levels early in infection with unchanged T cell recruitment

Since the Th1 response is pivotal for an adequate immune response against *M. tuberculosis*, we compared concentrations of IFNγ and IL-4 as prototypic Th1 and Th2 cytokine in lung homogenates and after splenocyte stimulation with the *M. tuberculosis* specific antigen PPD (table 2 and fig. 4). Moreover we studied T cell subsets in lungs and local lymph nodes at 2 and 5 weeks after infection (table II). Absence of SIGNR1 resulted in increased production of IFNγ and, although not significant, reduced production of IL-4 by splenocytes after 48 h of stimulation with PPD (fig. 4). No differences in local IFNγ and IL-4 concentrations were detected (table II). T cell subset recruitment was equal in lungs or draining lymph nodes of WT and SIGNR1 KO mice (table II). Nevertheless, the early activation status (% CD69 positive lymphocytes) of lung CD8+ T cells and draining lymph node CD4+ T cells was increased after 2 weeks but not after 5 weeks of infection in the SIGNR1 KO mice compared to the WT mice. The increase in IFNγ and T cell activation status in the SIGNR1 KO mice compared to WT mice indicate that SIGNR1 might be involved in the balance of the early immune response against *M. tuberculosis* towards Th2.

Similar cytokine and chemokine levels locally

It has been demonstrated that in vitro binding of *M. tuberculosis* ManLAM to the SIGNR1 homolog DC-SIGN expressed on DCs results in production of the anti-inflammatory cytokine IL-10 (5). We therefore assessed the local inflammatory response in WT and SIGNR1 deficient mice infected with *M. tuberculosis*. Despite the fact that ManLAM induced IL-10 production by DC in vitro, no differences in
local IL-10 production were observed during pulmonary tuberculosis in mice deficient for SIGNR1 compared to WT mice. As shown in table 2, no differences in total pulmonary cytokine and chemokine levels were observed: In addition to IL-10, TNF, IL-1β, KC and MIP-2 concentrations were comparable in WT and SIGNR1 KO mice (table III). Based on the cytokine profile measured locally, SIGNR1 is not involved in the induction of cytokine production to modulate the immune response.

<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>79 ± 3</td>
<td>29 ± 2</td>
<td>13 ± 2</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>SIGNR1 KO</td>
<td>73 ± 2</td>
<td>26 ± 3</td>
<td>17 ± 2</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>5 wks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>82 ± 3</td>
<td>29 ± 3</td>
<td>11 ± 3</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>SIGNR1 KO</td>
<td>88 ± 1</td>
<td>34 ± 3</td>
<td>8 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Lymph node</td>
<td></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>62 ± 2</td>
<td>10 ± 1</td>
<td>35 ± 2</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>SIGNR1 KO</td>
<td>63 ± 2</td>
<td>17 ± 3*</td>
<td>31 ± 2</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>5 wks</td>
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<td>87 ± 3</td>
<td>13 ± 3</td>
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<tr>
<td>SIGNR1 KO</td>
<td>83 ± 6</td>
<td>14 ± 1</td>
<td>10 ± 2</td>
<td>2 ± 1</td>
</tr>
</tbody>
</table>

Table II: Effect of SIGNR1 deficiency on T cell subsets in lungs during tuberculosis. T lymphocytes subsets in lungs or draining mediastinal lymph nodes of wild-type (WT) and SIGNR1 knock-out (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 per group. *P<0.05 versus WT mice.

Figure 4: Enhanced early antigen specific IFNγ response by splenocytes from infected SIGNR1 KO mice. Splenocytes were isolated from wild-type (WT; filled bars) and SIGNR1 knock-out mice (KO; open bars) 2 (A, C) and 5 (B, D) weeks after infection with 10⁵ Mycobacterium tuberculosis and stimulated with PPD for 48 h. IFNγ (A, B) and IL-4 (C, D) were measured in culture supernatants. Data are mean ± SEM of 6-8 mice per group per time point. *P < 0.05 versus WT mice.

Discussion

Early interactions between DC and M. tuberculosis are thought to be critical for mounting a protective anti-mycobacterial immune response. Recent studies have demonstrated that M. tuberculosis is able to bind to DC SIGN, a C-type lectin expressed on DC (5, 6). The interaction of M. tuberculosis cell wall component

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ManLAM and DC-SIGN resulted in suppression of LPS-induced DC maturation and in production of the immunosuppressive cytokine IL-10 in vitro (5). These data suggest that *M. tuberculosis* targets the C-type lectin DC-SIGN to suppress DC functions (5). In vivo studies are needed to fully understand the role of DC-SIGN in *M. tuberculosis* infection.

Five murine homologs of DC-SIGN have been identified in mice (11). Two of these homologs; mDC-SIGN and SIGNR1 are expressed by immune cells. mDC-SIGN is expressed by plasmacytoid pre-DC (15), whereas SIGNR1 is expressed by marginal zone macrophages of the spleen, lymph node medullary and subcapsular macrophages, liver sinusoidal endothelial cells (13) and by resident peritoneal macrophages (12).

Here, we have studied the role of SIGNR1, in a model of pulmonary tuberculosis. Although this homolog is capable of binding both *M. tuberculosis* and ManLAM, it is expressed by macrophages and not DC. IFN$_\gamma$ is the key protective cytokine in host defense against *M. tuberculosis* and IFN$_\gamma$ gene-deficient mice succumb to experimentally induced tuberculosis (22, 23). In SIGNR1 KO mice we expected that the absence of the ManLAM induced downmodulation would lead to an enhanced host defense response against *M. tuberculosis*. In contrast to our hypothesis, we were not able to detect any differences in pulmonary concentration of IL-10, IFN$_\gamma$ or other cytokines at 2 and 5 weeks post-infection.

Both CD4$^+$ and CD8$^+$ T cells contribute to host defense against *M. tuberculosis*. We therefore determined whether SIGNR1 plays a role in the activation of these cells during tuberculosis. No differences were detected between SIGNR1 KO and WT mice with respect to the numbers of CD4$^+$ and CD8$^+$ T lymphocytes at 2 and 5 weeks after infection. In addition T cell subsets did not differ in the local lymph nodes. Activated T cells are known to produce IFN$_\gamma$ and we found increased activation in pulmonary CD8$^+$ T cells and lymph node CD4$^+$ cells early after infection in SIGNR1 KO mice.

<table>
<thead>
<tr>
<th>ng/mL</th>
<th>2 wks</th>
<th>5 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN$_\gamma$</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>IL-10</td>
<td>2.2 ± 0.2</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>TNF</td>
<td>3.2 ± 0.7</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>IL-1$\beta$</td>
<td>2.2 ± 0.5</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>KC</td>
<td>4.7 ± 0.9</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>MIP-2</td>
<td>0.3 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

Table III: No effect of SIGNR1 deficiency on pulmonary cytokine and chemokine levels. Cytokine and chemokine levels (in ng/mL) in lung homogenates of WT and SIGNR1 KO mice 2 and 5 weeks after i.n. infection with $10^5$ CFU of *M. tuberculosis*. Data are mean ± SEM of 6-8 mice per group.
KO mice compared to the WT. However, the increased expression of CD69 in these cells did not lead to increased local levels of IFN\(\gamma\). Strikingly, at 2 weeks after infection splenocytes from SIGNR1 KO mice produced more IFN\(\gamma\) upon stimulation with the recall antigen PPD compared to WT mice. This indicates that the systemic immune response was enhanced at this early time-point in SIGNR1 KO mice compared to the WT confirming an immunosuppressive role for SIGNR1. This immunosuppressive role of SIGNR1 is confirmed by the trend that stimulated SIGNR1 KO splenocytes produced less IL4 compared to WT splenocytes. This indicates that SIGNR1 KO mice have a Th1 dominated immune response compared to a Th2 dominated immune response in the WT mice, suggesting a role for SIGNR1 in the Th1/Th2 balance of the immune response against \textit{M. tuberculosis}. However, the increased IFN\(\gamma\) production by splenocytes early in infection did not result in an enhancement of antibacterial response by SIGNR1 KO mice.

As a receptor for \textit{M. tuberculosis}, SIGNR1 could be involved in the binding and internalization of \textit{M. tuberculosis} for degradation. However, SIGNR1 is not expressed by alveolar macrophages and we did not observe any induction of SIGNR1 expression by alveolar macrophages or influx of SIGNR1 positive cells during infection, suggesting that SIGNR1 is not involved in the initial or late capture of \textit{M. tuberculosis} in the lung. Indeed, no differences were observed in the outgrowth of the bacteria in the tested organs.

Recent studies have demonstrated that SIGNR1 is involved in the early defense against \textit{Streptococcus (S.) pneumoniae} (17, 18). Lanoue et al. (17) demonstrated that, upon intraperitoneal infection of \textit{S. pneumoniae}, SIGNR1 expressed by peritoneal macrophages enhanced the clearance of \textit{S. pneumoniae} by phagocytosis. Recently, we have demonstrated that upon intranasal infection with \textit{S. pneumoniae}, SIGNR1 KO mice did not clear bacteria from lung and blood, and displayed severely enhanced inflammatory parameters compared to the WT mice (18). In addition, the natural antibody levels of anti-phosphorylcholine IgM did not increase during infection in SIGNR1 KO mice whereas the antibody level did increase during infection in the WT mice. These natural anti-phosphorylcholine IgM antibodies are produced by marginal zone B cells and are essential for protection against infection with \textit{S. pneumoniae}. Marginal zone macrophages have been suggested to capture antigens for presentation to marginal zone B cells. In our study we demonstrated that marginal zone macrophages from SIGNR1-deficient mice in contrast to wild-type mice are not able to capture pneumococci from blood, suggesting that SIGNR1 on marginal zone macrophages captures \textit{S. pneumoniae} for antigen presentation to and activation of marginal zone B cells resulting in an anti-phosphorylcholine IgM response. Hence, SIGNR1 seems to play a role in the early antibody mediated immune response against \textit{S. pneumoniae}. Host defense against \textit{M. tuberculosis} infection mainly depends on cell mediated
immunity (1, 24). Therefore, reduced natural antibodies due to the absence of SIGNR1 may reflect the small role of natural antibodies in host responses against *M. tuberculosis*.

There are several differences between human DC-SIGN and murine SIGNR1. Unlike DC-SIGN, SIGNR1 is abundantly expressed on splenic marginal zone, lymph node and resident peritoneal macrophages but not on DC (12, 13). Therefore, although the binding specificity of mDC-SIGN is unknown, it will be of interest to study the host response in mice deficient for mDC-SIGN, which is expressed by a DC subpopulation (14, 15).

This study reveals that SIGNR1 KO mice were equally susceptible to *M. tuberculosis* infection as WT mice as demonstrated by comparable antibacterial host defense, pulmonary inflammation and outcome of the disease. Therefore, our experiments did not provide proof for a crucial role of SIGNR1 in the immune response against *M. tuberculosis* although we have shown that in the early phase of infection, SIGNR1 modestly influences the Th1/Th2 balance of the immune response evoked by *M. tuberculosis*.

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**References**


