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Partial Redundancy of the Pattern Recognition Receptors, Scavenger Receptors, and C-Type Lectins for the Long-Term Control of Mycobacterium tuberculosis Infection

Nathalie Court,* Virginie Vasseur,* Rachel Vacher,* Cécile Frémont,* Yury Shebzukhov,† Vladimir V. Yeremeev,*,1 Isabelle Maillet,*, Sergei A. Nedospasov,†,‡ Siamon Gordon,§ Padraic G. Fallon,§ Hiroshi Suzuki,§ Bernhard Ryffel,* and Valérie J. F. Quesniaux*

Mycobacterium tuberculosis is recognized by multiple pattern recognition receptors involved in innate immune defense, but their direct role in tuberculosis pathogenesis remains unknown. Beyond TLRs, scavenger receptors (SRs) and C-type lectins may play a crucial role in the sensing and signaling of pathogen motifs, as well as contribute to M. tuberculosis immune evasion. In this study, we addressed the relative role and potential redundancy of these receptors in the host response and resistance to M. tuberculosis infection using mice deficient for representative SR, C-type lectin receptor, or seven transmembrane receptor families. We show that a single deficiency in the class A SR, macrophage receptor with collagenous structure, CD36, mannose receptor, specific ICAM-3 grabbing nonintegrin-related, or F4/80 did not impair the host resistance to acute or chronic M. tuberculosis infection in terms of survival, control of bacterial clearance, lung inflammation, granuloma formation, and cytokine and chemokine expression. Double deficiency for the SRs class A SR types I and II plus CD36 or for the C-type lectin mannose receptor plus specific ICAM-3 grabbing nonintegrin-related had a limited effect on macrophage uptake of mycobacteria and TNF response and on the long-term control of M. tuberculosis infection. By contrast, mice deficient in the TNF, IL-1, or IFN-γ pathway were unable to control acute M. tuberculosis infection. In conclusion, we document a functional redundancy in the pattern recognition receptors, which might cooperate in a coordinated response to sustain the full immune control of M. tuberculosis infection, in sharp contrast with the nonredundant, essential role of the TNF, IL-1, or IFN-γ pathway for host resistance to M. tuberculosis. The Journal of Immunology, 2010, 184: 7057–7070.
by M. tuberculosis lipoarabinomannan through engagement of the human mannose receptor (16, 17). The human C-type lectins man-
nose receptor and DC-specific ICAM-3–grabbing nonintegrin (DC-
SIGN) were also implicated in the negative regulation of TLR-
induced responses by mannose-capped lipoarabinomannan (18,
19) through Raf-1 kinase-dependent activation and IL-10 secretion,
leading to inhibition of Th1-polarized responses (20, 21).

The SR family includes two members in the A subclass that are
expressed on lung macrophages and DCs: macrophage receptor
with collagenous structure (MARCO) and class A SR types I and II
(referred to herein as SR-A) (22–24). MARCO and SR-A have a
collagenous structure, bind acetylated low-density lipoprotein
and bacteria, are expressed on alveolar macrophages, and promote
the uptake and clearance of inhaled particles and bacteria (25–
28). MARCO expression is induced on alveolar macrophages
after BCG infection (29). Moreover, MARCO was shown recently
to tether M. tuberculosis trehalase 6,6′-dimycolate (cord factor)
to macropores and activate the TLR2/CD14 signaling pathway
(30). The contribution of SR-A to this response was more limited
(30); it was reported to downmodulate alveolar proinflammatory cy-
tokine responses to cord factor (31). The roles of SR-A and MARCO
in the in vivo response to mycobacteria have not been reported.

The class B SR CD36 was shown to be required for the uptake
of mycobacteria in Drosophila macrophage-like cells (32). CD36
cooperates with TLR2 in sensing bacteria and bacterial ligands,
acting as a coreceptor for the induction of proinflammatory cyto-
kines; CD36-deficient mice were unable to control Staphylococcus
aureus infection (33, 34). Because TLR2 is one of the TLRs
most involved in mycobacterial motives recognition, it was of
interest to see whether CD36 might also contribute to the TLR2-
mycobacterial response.

C-type lectins involved in the recognition of mycobacteria include
the mannose receptor and human DC-SIGN family (35, 36). Seven
DC-SIGN homolog genes and a pseudogene have been described
in mice (37, 38). In this study, we concentrated on addressing the role
of murine mannose receptor and specific ICAM-3 grabbing non-
integrin related (SIGNR1), because SIGNR1 was reported to asso-
ciate with TLR4/myeloid-differentiation factor 2 and modulate
downstream signaling under specific conditions (39).

The seven transmembrane receptor, epidermal growth factor
module-containing mucin-like hormone receptor 1 (EMR1) murine
ortholog F4/80 was also studied. Indeed, F4/80 is upregulated in
differentiated macrophages, and it may be implicated in macro-
phage adhesion and migration (40). F4/80 macrophage expression
was shown to be reduced after Mycobacterium bovis BCG infection
(41). F4/80 was implicated in macrophage-dependent modulation of
IFN-γ release by NK cells in response to Listeria (42), and it was
shown to be required for the induction of Ag-specific effector reg-
ulatory T cells in peripheral tolerance (43).

Little is known about the relative role and potential redundancy of
these receptors in the host immune response to acute M. tuberculosi-
us infection. In this study, we addressed this question using genetic
deficient mice for a selection of receptors representa-
tive of SR, C-type lectin receptor, or seven transmembrane
receptor families. We show that single deficiency in MARCO,
SR-A, CD36, mannose receptor, SIGNR1, or F4/80 or double
deficiency for the SRs SR-A plus CD36 or for the C-type lectins
mannose receptor plus SIGNR1 did not impair the control of acute
or chronic M. tuberculosis infection. Mice deficient for proinflam-
matory cytokine pathways, such as TNF, IL-1R1, the adaptor
MyD88, or IFN-γ, were tested in parallel as internal controls for
the drastic phenotypes obtained when essential signaling pathways
are not functional. The data point to a high redundancy in the PRRs
tested for the control of M. tuberculosis infection, whereas there is
no such redundancy in the proinflammatory TNF, IL-1, or IFN-γ
cytokine pathways.

Materials and Methods

Mice

Mice deficient for mannose receptor (44), MARCO (26), SR-A (45), CD36 (46), SR-A plus CD36 (47), F4/80 (43), SIGNR1 (48), TNF (49), IL-1R1 (50), or MyD88 (51) were bred in our animal facility at the Transgenose
Institute (National Center for Scientific Research, Orleans, France). All
mice were backcrossed ≥7–10 times on the C57BL/6 genetic background.
For experiments, 8–15-wk-old animals were kept in isolators in a biosafety
animal unit. The infected mice were monitored regularly for clinical status
and weighed weekly. All animal experimental protocols were approved by
the Regional Ethics Committee for Animal Experimentation, section “Cen-
tre-Limousin” (No. CL2008-011).

Infection

M. tuberculosis H37Rv (Pasteur Institute, Paris, France) aliquots kept
frozen at −80°C were thawed, briefly vortexed, and diluted in sterile saline
containing 0.05% Tween 20; clipping was disrupted by 20 repeated
aspirations through a 20-gauge needle (Omnican, Braun, Germany). Pul-
monary infection with M. tuberculosis H37Rv was performed by delivering
~200 bacteria into the nasal cavities (20 μl each) under xylazine-
ketamine anesthesia; the inoculum size was verified 24 h postinfection
by determining bacterial load in the lungs.

Bacterial load in tissues

Bacterial loads in the lung of infected mice were evaluated at different
time points postinfection with M. tuberculosis H37Rv, as described (52). Organs
were weighed, and defined aliquots were homogenized in 0.05% Tween 20
NaCl. Ten-fold serial dilutions of organ homogenates were plated in dup-
licate onto Middlebrook 7H11 agar plates containing 10% oleic acid/
albumin/dextrose/catalase and incubated at 37°C. Colonies were enumerated
at 3 wk, and results were expressed as log10 CFU per organ.

Pulmonary cytokine concentrations

Cytokine and chemokine concentrations in the lung of infected mice were
evaluated in lung homogenates after passage through a 0.20-μm filter using
Endogen Search Light protein array by Perbio Thermo Fisher Scientific
(Woburn, MA).

Histopathological analysis

For histological analysis, lungs were removed at different time points
of infection, fixed in 10% phosphate-buffered formalin, and embedded in par-
affin. Two- to 3-μm sections were stained with H&E and a modified Ziehl-
Neelsen (ZN) method. The latter involved staining in a prewarmed (60°C)
carbol-fuchsin solution for 10 min, followed by destaining in 20% sulfuric
acid and 90% ethanol, before counterstaining with methylene blue. Free
airway space, lung cellular infiltration, edema, bacilli burden, and necrosis
were quantified, using a semiquantitative score with increasing severity of
changes (0–5), by two independent observers, including a trained pathol-
ologist (B.R.).

Primary macrophage cultures

Nonelicited peritoneal macrophages were collected after 0.34 M sucrose
lavage. Bone marrow cells were isolated from femurs and dif-
morphinated into macrophages after culturing at 106 cells/ml for 7 d in DMEM
(Sigma-Aldrich, St; Louis, MO) supplemented with 20% horse serum and
30% L929 cell-conditioned medium as a source of M-CSF (53). Three days
after washing and reculturing in fresh medium, the cell preparation contained
a homogeneous population of macrophages. Macrophages were plated in
96-well microculture plates (at 104 cells/well in DMEM supplemented
with 10% FCS and 10% ethanol, before counterstaining with methylene blue. Free
airway space, lung cellular infiltration, edema, bacilli burden, and necrosis
were quantified, using a semiquantitative score with increasing severity of
changes (0–5), by two independent observers, including a trained pathol-
ologist (B.R.).
M. tuberculosis H37Rv (heat-killed for 40 min at 80°C; two bacteria/cell). Cell supernatants were harvested after 24 h of stimulation in the presence of IFN-γ (100 U/ml) for TNF quantification using commercial ELISA (R&D Systems, Minneapolis, MN).

**Confocal microscopy of mycobacteria internalization**

Macrophage monolayers were established by plating 10³ cells in 0.2 ml DMEM, as described above, onto sterile glass coverslips and incubating them overnight at 37°C in humidified air containing 5% CO₂. BCG-GFP (kind gift from V. Snewin, London, U.K.) was added to the cultures at an MOI of 1. After 2 h at 37°C under a humidified atmosphere containing 5% CO₂, the medium was removed and fixed with paraformaldehyde 4% in PBS for 20 min at 37°C or overnight at 4°C. After fixation, macrophages on coverslips were washed once in warm PBS for 10 min. Cells were permeabilized for 3 min with 0.1% Triton X-100 in PBS, washed 10 min with PBS, quenched with 50 mM NH₄Cl for 30 min, washed 10 min with PBS, and incubated for 30 min with 1% BSA in PBS, and washed again in PBS. To stain F-actin, macrophages were incubated for 20 min with β-phallolidin conjugated to rhodamine at 5 U/ml (Molecular Probes, Eugene, OR), followed by two 5-min washes in PBS. Coverslips were mounted using DAKO mounting medium with DAPI. BCG-GFP internalization was assessed using a fluorescence Leica DM IRBE microscope (Leica, Reuil Malmaison, France; ×100 oil immersion objective) by counting the macrophages containing one or two isolated intracellular bacteria and the noninfected macrophages in 10 fields of view per slide (three or four slides per group).

**Analysis of RPR gene expression**

Total RNA was isolated from lungs by TRIzol reaction (Sigma-Aldrich) and purified using an RNeasy Mini Kit (Qiagen, Valencia, CA) at the indicated times after M. tuberculosis infection. For quantitative RT-PCR, 1 µg total RNA was pretreated by DNaseI and converted to cDNA by ImProm-II Reverse Transcriptase (Promega, Madison, WI) using random nonamer primers. Quantitative PCR reactions were performed using the Brilliant II SYBR QPCR kit and Stratagene Mx3005P thermocycler (Agilent, Palo Alto, CA). The following program was used: 95°C for 15 min and 40 cycles at 95°C for 10 s and 60°C for 30 s.

Primers used for quantitative PCR included β-actin forward primer: 5'-CTCTTGAATGCAATCTCTG-3', β-actin reverse primer: 5'-TAAAAACAGCCTAGTCAAACCTG-3'; SA-1/MsI1 forward primer: 5'-TGGACGACATGATTCTA-3'; SA-1/MsI1 reverse primer: 5'-CTATTTGATAGCATATGAC-3'; CD36 forward primer: 5'-TCAGATCCGCAACACAGCT-3'; Mrc1 forward primer: 5'-TGTGTTGGATGCTGGAAGG-3'; Mrc1 reverse primer: 5'-TCAGATTCTTTGCAGTTCAAG-3'; Mrc1 forward primer: 5'-CTATTTGATAGCATATGAC-3'; Mrc1 reverse primer: 5'-CTATTTGATAGCATATGAC-3'; F4/80–Emr1 forward primer: 5'-CTATTTGATAGCATATGAC-3'; F4/80–Emr1 reverse primer: 5'-CTATTTGATAGCATATGAC-3'; and F4/80–Emr1 reverse primer: 5'-CTATTTGATAGCATATGAC-3'.

**Statistical analysis**

Analysis was performed using the Student t test and two-way ANOVA, with the Bonferroni posttest; p values ≤0.05 were considered significant.

**Results**

**Controlled acute M. tuberculosis infection in the absence of SRs SR-A or MARCO**

Because the SRs MARCO and SR-A are expressed on alveolar macrophages and promote the uptake and clearance of inhaled bacteria (25–27), and class B SR CD36 cooperates with TLR2 in sensing bacteria, we first addressed the role of these receptors in the response to acute M. tuberculosis infection. Mice deficient for SR-A subclass (MARCO or SR-A) were infected with M. tuberculosis. All groups survived and gained body weight during the first 3 mo postinfection, in contrast to mice deficient for IL-1R1, which rapidly lost weight and had to be killed within 4 wk of infection, or to mice deficient for IFN-γR, which lost weight and eventually succumbed at 9 wk postinfection (Fig. 1A). Although sensitive mice deficient for IL-1R1 or IFN-γR exhibited >1 log₁₀ greater bacterial load in the lungs compared with wild-type (WT) controls 3–7 wk postinfection (Fig. 1B, 1C), SR-A− and MARCO-deficient mice had bacterial loads similar to WT mice in the lung up to 13–16 wk postinfection (Fig. 1B, 1C). Increased lung weight, a sign of marked inflammation, is usually seen in IL-1R1− or IFN-γR-deficient mice; SR-A− or MARCO-deficient mice had a lung weight similar to the WT controls up to 13–16 wk postinfection (Fig. 1D, 1E), indicating no obvious organ inflammation.

Granuloma formation, the result of a structured cell-mediated immune response, is crucial for controlling mycobacterial growth. We next examined lung morphology and asked whether the SRs are essential for granuloma formation upon M. tuberculosis infection. Macroscopically, the lungs of SR-A− or MARCO-deficient mice displayed no obvious difference from WT controls, in sharp contrast to the lungs of IL-1R1− or IFN-γR−deficient mice, which displayed large subpleural and confluent nodules at 3–7 wk postinfection (Fig. 1F, 1G). Microscopic investigation of the lungs of SR-A− or MARCO-deficient mice revealed granuloma formation, characterized by the accumulation of macrophages accompanied by lymphocytic perivascular and peribronchiolar cuffing, similar to WT mice (Fig. 1H–K). In contrast, IL-1R1− or IFN-γR−deficient mice had severe inflammation, with significant reduction of ventilated alveolar spaces and massive mononuclear and neutrophil infiltration with extensive confluent necrosis in the absence of proper granuloma formation (Fig. 1A). Scattered intracellular mycobacteria were present in the lung of SR-A− and MARCO-deficient mice, similar to WT mice, in contrast to the abundant mycobacteria observed extracellularly in the lung of IL-1R1− and IFN-γR−deficient mice (Fig. 1L–O). Thus, the absence of SR-A or MARCO did not affect the host response to acute M. tuberculosis infection; the animals survived, controlled the infection, and displayed appropriate granulomatous responses, with no excessive inflammation of the lungs.

**Controlled acute M. tuberculosis infection in the absence of the C-type lectin mannose receptor or SIGNIR1**

Because both C-type lectins (mannose receptor and DC-SIGN) are involved in the human response to mycobacteria and may contribute to the immunomodulation of the host response by mycobacteria, we next addressed the role of the murine receptors in the response to acute M. tuberculosis infection. Mice deficient for SIGNIR1 or mannose receptor infected with M. tuberculosis survived the loss of body weight similar to WT mice during the first 3 mo postinfection (Fig. 2A), in contrast to mice deficient for TNF or IL-1R1, which succumbed within 3–4 wk of infection. Although mice deficient for IL-1R1 or TNF exhibited pulmonary bacterial loads 1–3 log₁₀ greater than WT controls 4 wk postinfection, SIGNIR1- or mannose receptor-deficient mice controlled the infection as well as WT mice for the first 8–12 wk of the experiment (Fig. 2B, 2C). They showed lung weights similar to WT mice at 2–3 mo of infection, whereas IL-1R1− or TNF−deficient mice had increased lung weight, indicative of marked lung inflammation at 4 wk postinfection (Fig. 2D, 2E). Macroscopically, the lungs of SIGNIR1- or mannose receptor-deficient mice were essentially similar to those of WT mice, whereas large and numerous confluent nodules occurred in IL-1R1− or TNF−deficient mice at 4 wk of infection (Fig. 2F, 2G). Although SIGNIR1-deficient mice showed similar lung weight to WT controls, histologically they presented increased lung cell infiltration and edema at 4 wk, a time point at which extensive necrotic lesions had developed in susceptible IL-1R1−deficient mice (Fig. 2H, 2I). In addition, lungs of SIGNIR1-deficient mice displayed some accumulation of mycobacteria, albeit not as marked as the uncontrolled bacilli growth seen in IL-1R1−deficient mice, whereas only scattered isolated bacilli were found in WT lungs.
FIGURE 1. Controlled acute *M. tuberculosis* infection in the absence of the SRs MARCO or SR-A. A. Mice deficient for MARCO, SR-A, IFN-γR, or IL-1R1 and WT mice were exposed to aerogenic *M. tuberculosis* H37Rv and monitored for body weight. Mean values of \( n = 8–15 \) mice per group from two or three experiments with \( n = 3–4 \) cytokine pathway-deficient control mice. Significant body weight change was found after week 3 for IL-1R1 knockout (KO) and week 4 for IFN-γR KO mice, which rapidly lost weight and succumbed, and after weeks 3 and 9 for SR-A KO and MARCO KO mice, respectively.
Comparing with WT control, three groups that survived and gained body weight during the first 3 mo postinfection. The number of viable bacteria present in the lungs (B, C) and the lung relative weight (D, E) were measured at 3–16 wk postinfection. Mean ± SD of n = 4 representative of one of two independent experiments. **p ≤ 0.01; ***p ≤ 0.001. Macrophage examination of the postinfection (F) and of MARCO- and IFN-γR-deficient mice and WT extensive inflammation and necrosis in infected IL-1R1-deficient lungs and SIGNR1-deficient mice are shown in Figure 2F. Compared with WT control, three groups that survived and gained body weight during the first 3 mo postinfection. The number of viable bacteria present in the lungs (B, C) and the lung relative weight (D, E) were measured at 3–16 wk postinfection. Mean ± SD of n = 4 representative of one of two independent experiments. **p ≤ 0.01; ***p ≤ 0.001. Macrophage examination of the postinfection (F) and of MARCO- and IFN-γR-deficient mice and WT extensive inflammation and necrosis in infected IL-1R1-deficient lungs and SIGNR1-deficient mice are shown in Figure 2F. Compared with WT control, three groups that survived and gained body weight during the first 3 mo postinfection. The number of viable bacteria present in the lungs (B, C) and the lung relative weight (D, E) were measured at 3–16 wk postinfection. Mean ± SD of n = 4 representative of one of two independent experiments. **p ≤ 0.01; ***p ≤ 0.001. Macrophage examination of the postinfection (F) and of MARCO- and IFN-γR-deficient mice and WT extensive inflammation and necrosis in infected IL-1R1-deficient lungs and SIGNR1-deficient mice are shown in Figure 2F. Compared with WT control, three groups that survived and gained body weight during the first 3 mo postinfection. The number of viable bacteria present in the lungs (B, C) and the lung relative weight (D, E) were measured at 3–16 wk postinfection. Mean ± SD of n = 4 representative of one of two independent experiments. **p ≤ 0.01; ***p ≤ 0.001. Macrophage examination of the postinfection (F) and of MARCO- and IFN-γR-deficient mice and WT extensive inflammation and necrosis in infected IL-1R1-deficient lungs and SIGNR1-deficient mice are shown in Figure 2F. Compared with WT control, three groups that survived and gained body weight during the first 3 mo postinfection. The number of viable bacteria present in the lungs (B, C) and the lung relative weight (D, E) were measured at 3–16 wk postinfection. Mean ± SD of n = 4 representative of one of two independent experiments. **p ≤ 0.01; ***p ≤ 0.001. Macrophage examination of the postinfection (F) and of MARCO- and IFN-γR-deficient mice and WT extensive inflammation and necrosis in infected IL-1R1-deficient lungs and SIGNR1-deficient mice are shown in Figure 2F. Compared with WT control, three groups that survived and gained body weight during the first 3 mo postinfection. The number of viable bacteria present in the lungs (B, C) and the lung relative weight (D, E) were measured at 3–16 wk postinfection. Mean ± SD of n = 4 representative of one of two independent experiments. **p ≤ 0.01; ***p ≤ 0.001. Macrophage examination of the postinfection (F) and of MARCO- and IFN-γR-deficient mice and WT extensive inflammation and necrosis in infected IL-1R1-deficient lungs and SIGNR1-deficient mice are shown in Figure 2F.
FIGURE 2. Controlled acute *M. tuberculosis* infection in the absence of the C-type lectins SIGNR1 and mannose receptor. A. Mice deficient for SIGNR1, mannose receptor, TNF, or IL-1R1 and WT mice were exposed to *M. tuberculosis* H37Rv and monitored for body weight. Mean values of *n* = 8–15 mice per...
and chemokine concentrations were essentially normal in these mice (Fig. 5). Macroscopically, lungs of SR-A plus CD36-deficient mice presented large nodules (Fig. 6D), and histological sections revealed reduced free air space, with more cells infiltrating the lungs and edema, compared with WT controls at 1 mo (Fig. 6F). However, both groups presented similar lung morphology 9 mo postinfection (Fig. 6G), and no necrotic area could be detected as in the TNF-deficient mice.

In parallel, we infected mice doubly deleted for mannose receptor plus SIGNR1 with M. tuberculosis and monitored their body weight up to 5 mo. Mice lacking both lectins were able to control the infection with no loss in body weight, except for 2 of 13 mice who lost weight and had to be killed on days 69 and 98 (Fig. 7A). Doubly deficient mice showed no impairment of bacterial growth control, as seen at 1, 2, and 5 mo postinfection (Fig. 7B), and no obvious inflammation of the lungs (Fig. 7C), whereas mice lacking the mannose receptor lost weight and died at this time point. In contrast, SIGNR1-deficient mice presented increased cell infiltration in lungs with edema compared with WT mice, whereas mannose receptor-deficient mice exhibited well-defined granuloma at 4 wk (H, I, K), 8 wk (J), or 12 wk (L), with few mycobacteria at these time points (M–P). I–L, H&E, original magnification ×100. M–P, ZN staining, original magnification ×1000. Arrows point to ZN positive bacilli.
FIGURE 4. Chronic *M. tuberculosis* infection is controlled in SIGNR1-, MARCO-, or F4/80-deficient mice. A, Mice deficient for SIGNR1, MARCO, or F4/80 and WT mice were exposed to aerogenic *M. tuberculosis* H37Rv and monitored for body weight (mean values of *n* = 6–12 mice per group). No
and mice deficient in mannose receptor plus SIGNR1 (Fig. 7B). There was no difference in bacterial load or lung weight between WT controls and mice lacking both receptors exhibited a slight increase in pulmonary cell infiltration compared with WT controls, as well as visible mycobacteria (Fig. 7G, 7H), whereas bacilli were barely detectable in WT controls (data not shown).

Therefore, the double deficiency in SRs (SR-A plus CD36) or in both C-type lectins (mannose receptor plus SIGNR1) did not result in a systematic impairment of the long-term control of *M. tuberculosis* infection.

**Competence of PRR-deficient macrophages for TNF release in response to mycobacteria**

TNF plays an important role in the control of local immune response to intracellular pathogens, such as *M. tuberculosis*. Therefore, we investigated the ability of PRR-deficient resident peritoneal macrophages or bone marrow-derived macrophages to secrete TNF in response to mycobacteria in vitro (Fig. 8). Peritoneal macrophages deficient for MARCO or SR-A produced levels of TNF comparable to WT macrophages after stimulation with *M. bovis* BCG or *M. tuberculosis* H37Rv (Fig. 8A). In contrast, macrophages lacking SR-A plus CD36 exhibited a slight decrease in TNF production in response to mycobacteria, which was also seen, to a lower level, with CD36 deficiency (Fig. 8A). As expected, macrophages deficient for CD36 did not respond to MALP2, that interacts and signals through TLR2 and CD36, whereas they responded normally to Pam3CSK4, an agonist of TLR2/TLR1 heterodimers (Fig. 8B).

Similarly, resident peritoneal macrophages deficient in SIGNR1 or doubly deficient for mannose receptor plus SIGNR1 produced TNF in response to mycobacteria, albeit at slightly reduced levels in the latter cells (Fig. 8C). Deficiency in F4/80 had no effect on the TNF response of bone marrow-derived macrophages to mycobacteria (Fig. 8D). Comparable results were obtained for the production of NO, an important mycobactericidal mediator, in all macrophages tested (data not shown). Therefore, none of the PRRs tested was essential for the in vitro TNF response of macrophages stimulated with mycobacteria, but they might cooperate to result in a full inflammatory response.

**Internalization of BCG into macrophages from PRR-deficient mice**

Several PRRs, including C-type lectins and complement receptors, have been implicated in the recognition and binding of *M. tuberculosis* in human cells (35, 36). Therefore, we asked whether murine homologs are important for the internalization of *Mycobacterium bovis* BCG. We showed previously that the absence of TLR2 plus TLR4 or of MyD88 led to a reduction in BCG-GFP internalization by one third or one half, respectively (54). Macrophages lacking SIGNR1, mannose receptor, mannose receptor plus SIGNR1, MARCO, SR-A, CD36, or SR-A plus CD36 were incubated with BCG-GFP for 2 h, and the proportion of macrophages infected with one or two mycobacteria was analyzed using confocal microscopy. Macrophages from MARCO−/−, SR-A−/−, or CD36-deficient mice internalized mycobacteria at a similar rate to WT controls, whereas in the absence of SR-A and CD36, there was a decrease in BCG internalization by resident peritoneal (Fig. 8E) and bone marrow-derived macrophages (Fig. 8F), similar to what was seen in the absence of MyD88 (46% reduction; data not shown) and mice deficient in mannose receptor plus SIGNR1 (Fig. 7B).

**Tuberculosis in PRR-deficient mice**

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shown). In contrast, deficiency in SIGNR1 or mannose receptor plus SIGNR1 led to a slight increase in BCG uptake by peritoneal macrophages (Fig. 8G), whereas bone marrow-derived macrophages from mannose receptor-deficient mice presented a similar internalization rate to WT cells (Fig. 8H). Therefore, the absence of any one of the receptors tested did not compromise BCG internalization, whereas the absence of SR-A plus CD36 resulted in a reduced internalization.

Discussion

Several types of PRRs have been identified in sensing, binding, or signaling M. tuberculosis motives, including TLRs, nod-like receptors, complement receptors, SRs, and C-type lectin receptors. An in vitro study indicated that blocking complement and mannose receptors did not completely abrogate binding of M. tuberculosis to human macrophages (2). Further blocking of class A SRs abrogated nearly all binding, indicating that they are important mediators of M. tuberculosis–macrophage interactions (2). In this study, we attempted a comparative study of receptors of the different classes of PRRs to assess their importance in the control of an acute or chronic M. tuberculosis infection.

The role of receptors representative of SR, C-type lectin receptor, or seven transmembrane receptor families in the host response to acute and chronic M. tuberculosis infection was studied using genetically deficient mice. We and other investigators showed previously that although mycobacteria produce agonist molecules able to trigger TLR2, TLR4, and TLR9, the role of these receptors in the control of in vivo M. tuberculosis infection is limited (10, 12, 14). Therefore, it is essential to understand the involvement of
the other PRRs in mounting the host innate and adaptive responses and in controlling *M. tuberculosis* infection.

Expression of MARCO and SR-A is rapidly induced on macrophages after BCG infection, and a role for these SRs in the host antibacterial defense was suggested (29, 55). SR-A expression was increased in the lung of *M. tuberculosis*-infected mice 2–4 wk postinfection in our model, as revealed by microarray analysis (data not shown) and confirmed by quantitative PCR (Supplemental Fig. 1). This is in agreement with the strong SR-A upregulation reported 3–9 wk after *M. tuberculosis* infection in mice (56), as well as in human pulmonary macrophages from tuberculosis patients (57). However, MARCO expression was not affected 1–4 wk after *M. tuberculosis* infection in our model (Supplemental Fig. 1). MARCO was recently shown to be essential for *M. tuberculosis* cord factor recognition and activation of the TLR2/CD14 signaling pathway by macrophages (30), whereas the contribution of SR-A to this response was more limited and associated with TLR2 and TLR4 (30). In this study, although mycobacterial aggregates seemed slightly more prominent in MARCO-deficient mice, we showed no impairment in the control of acute or chronic *M. tuberculosis* infection in the absence of MARCO or SR-A.

Decreased expression of the class B SR CD36 was reported on monocytes isolated from pulmonary, pleural, or miliary tuberculosis patients or after in vitro infection with *M. tuberculosis* (58). In our
model, CD36 expression in the lung was slightly decreased 2–4 wk after *M. tuberculosis* infection, as revealed by microarray analysis (data not shown) and confirmed by quantitative PCR (Supplemental Fig. 1). The inverse regulation of SR-A and CD36 expression after *M. tuberculosis* infection might also contribute to the functional compensation between the SRs. CD36 cooperates with TLR2 in sensing bacteria, acting as a coreceptor for the induction of proinflammatory cytokines (33). Because TLR2 is one of the TLRs most involved in mycobacterial motives recognition, it was of interest to see whether CD36 might also contribute to the TLR2–mycobacterial response. The absence of SR-A plus CD36 partially impaired macrophage internalization of BCG and TNF release in response to mycobacteria. In vivo, the absence of SR-A plus CD36 seemed to slightly compromise the control of *M. tuberculosis* growth and inflammatory response in the lungs 4 wk postinfection, which normalized thereafter for up to 9 mo of infection. However, the long-term control of *M. tuberculosis* infection was compromised because three mice had to be terminated 5–9 mo postinfection. These phenotypes were clearly not as marked as those seen in the absence of cytokines, such as TNF or IL-1 pathways, but they suggest that the contribution of several receptor families might be necessary for full, long-term control of the infection.

The human C-type lectins mannose receptor and DC-SIGN are involved in the recognition of mycobacteria, and they were implicated in the negative regulation of TLR-induced response, likely contributing to *M. tuberculosis* immune-evasion strategies (35, 36). Among the seven murine SIGNR homologs, the absence of SIGNR1, SIGNR3, or SIGNR5 did not hamper survival after *M. tuberculosis* infection (59, 60). In this study, we concentrated on addressing the role of murine mannose receptor and SIGNR1, because SIGNR1 may associate with TLR4/myeloid-differentiation factor 2 and module downstream signaling (39). Mannose receptor expression was decreased in the lung of *M. tuberculosis*-infected mice 3 wk postinfection in our model, as revealed by microarray analysis (not shown) and confirmed by quantitative PCR (Supplemental Fig. 1), whereas SIGNR1 expression was very low and decreased further on day 21 postinfection. Human mannose receptor was implicated in the phagocytosis of mycobacteria by macrophages (16, 17), but we did not see any effect of the deficiency in the murine mannose receptor on BCG uptake in vitro. However, macrophages lacking SIGNR1 or both C-type lectins (mannose receptor and SIGNR1) showed a slightly increased macrophage uptake of BCG, which was associated with a reduced production of TNF in vitro. In vivo, mice deficient for mannose receptor plus SIGNR1 seemed to have a more marked pathology 5 mo postinfection, with high cell infiltration and mycobacteria foci in the lung; one mouse each had to be killed at 2 and 4 mo of infection. Therefore, there was a slight phenotype in the absence of mannose receptor in combination with SIGNR1; overall, this did not impair the resistance to acute or chronic *M. tuberculosis* infection, excluding an essential role of receptor family members during chronic infection. This finding suggests that human-like receptor families might be necessary for full, long-term control of *M. tuberculosis* infection.
involvement of either receptor and suggesting a functional redundancy of both receptors for the host response to *M. tuberculosis*.

F4/80 was proposed to be involved in cell adherence and migration because it is expressed at a low level on circulating monocytes, whereas it is upregulated in differentiated macrophage populations (40). F4/80 expression was shown to be downregulated on peritoneal macrophages after local *M. bovis* BCG infection (41), which may be compatible with an increased migration upon infection. Although no significant change in pulmonary F4/80 expression was noted 1–2 wk after airway *M. tuberculosis* infection, a trend for higher expression was seen at day 21 by quantitative PCR, and a sharp increase was noted on day 28 (Supplemental Fig. 1). Y. Shebzukhov and S.A. Nedospasov, unpublished observations. Although Abs to F4/80 revealed a functional requirement for F4/80 in the production of TNF, IL-12, or IFN-γ after splenocyte exposure to *Listeria monocytogenes* (42), no phenotype was reported in F4/80-deficient mice (43), with the exception of a role for F4/80 in the generation of efferent CD8+ regulatory T cells (43). Little was known of the potential role for F4/80 in the host response to *M. tuberculosis*. We showed in this study that F4/80 is dispensable for macrophage TNF response to mycobacteria and that the absence of F4/80 does not compromise the control of acute and chronic *M. tuberculosis* infection.

Several of the receptors studied were reported to modulate the cytokine response mediated by TLR receptors, such as C-type lectins downregulating TLR4-induced IL-12 responses (18, 20), CD36 acting as a TLR2 coreceptor for the induction of proinflammatory cytokines (33), or SR-A and MARCO distinctly regulating IL-12 release (62). TLR2 is essential in mediating the response to killed mycobacteria and to several molecularly identified mycobacterial Ags, although it does not seem indispensable for the in vitro response to live mycobacteria (63), raising the question about whether other receptors might be involved in this response. The release of TNF, a cytokine central to the control of mycobacterial infection, was largely unaffected in macrophages deficient for MARCO, SR-A, CD36, F4/80, SIRN1, or mannose receptor upon mycobacterial stimulation. A partial reduction in TNF release was observed in macrophages doubly deficient for SR-A plus CD36 or for mannose receptor plus SIRN1. Additionally, the pattern of pulmonary expression of IL-12p40, -12p70, and -23; IFN-γ; IL-1α, -1β, and -19a; TNF-α; and the chemokines CCL2 (JE/MPC-1) and CXCL1 (KC) was not drastically affected after 9 mo of *M. tuberculosis* infection in mice deficient for SIRN1, MARCO, F4/80, or SR-A plus CD36. Therefore, none of the single PRRs tested was essential for the in vivo TNF response of macrophages stimulated with mycobacteria or for the in vivo lung cytokine and chemokine expression in late infection, but they might cooperate to provide a full inflammatory response.

The essential role of several proinflammatory cytokine pathways, such as TNF, IL-1R1, the adaptor MyD88, or IFN-γ, although already well documented (4–9), was reassessed in the current study. The cytokine pathway-deficient mice served as internal controls in each experiment, illustrating the type of extreme phenotype that can be expected when an essential signaling pathway is not functional, as well as controlled for the reproducibility and virulence of the *M. tuberculosis* infections in the different experiments. More importantly, we showed that abrogation of several proinflammatory cytokine pathways leads to similarly drastic phenotypes, confirming that none of the cytokines can compensate for the other, whereas the disruption of several PRRs leads only to moderate phenotypes. Thus, the redundancy seems much greater at the level of PRRs than at the level of the downstream cytokine pathways activated.

In this study, we documented a functional redundancy in the different PRRs, in sharp contrast to the proinflammatory cytokine TNF, IL-1, and IFN-γ pathways, which are each essential and cannot compensate for each other in the control of *M. tuberculosis* infection. Although most cytokines are recognized by a unique or a limited set of receptors, mycobacteria express multiple molecular motives recognized by multiple receptors and susceptible to triggering the activation of different signaling pathways. Our results suggest that different receptors or receptor families might cooperate in a coordinated response to sustain the full immune control of *M. tuberculosis* infection.

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**Disclosures**

The authors have no financial conflicts of interest.

**References**


