Damping Excessive Inflammation and Tissue Damage in *Mycobacterium tuberculosis* Infection by Toll IL-1 Receptor 8/Single Ig IL-1-Related Receptor, a Negative Regulator of IL-1/TLR Signaling

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Damping Excessive Inflammation and Tissue Damage in Mycobacterium tuberculosis Infection by Toll IL-1 Receptor 8/Single Ig IL-1-Related Receptor, a Negative Regulator of IL-1/TLR Signaling

Cecilia Garlanda,† Diana Di Liberto,† Annunciata Vecchi,† Marco P. La Manna,* Chiara Buracchi,† Nadia Caccamo,* Alfredo Salerno,* Francesco Dieli,2,3* and Alberto Mantovani2†

Toll IL-1R 8/single Ig IL-1-related receptor (TIR8/SIGIRR) is a member of the IL-1R family, expressed by epithelial tissues and immature dendritic cells, and is regarded as a negative regulator of TLR/IL-1R signaling. TIR8-deficient mice were rapidly killed by intranasal administration of low doses of Mycobacterium tuberculosis, despite controlling efficiently the number of viable bacilli in different organs. TIR8−/−-infected mice showed an increased number of neutrophils and macrophages in the lungs; however, mycobacteria-specific CD4 and CD8 T cells were similar in TIR8−/− and TIR8+/+ mice. Exaggerated mortality of TIR8−/− mice was due to massive liver necrosis and was accompanied by increased levels of IL-1β and TNF-α in lung mononuclear cells and serum, as well as by increased production of IL-1β and TNF-α by M. tuberculosis-infected dendritic cells in vitro. Accordingly, blocking IL-1β and TNF-α with a mix of anti-cytokine Abs, significantly prolonged survival of TIR8−/− mice. Thus, TIR8/SIGIRR plays a key role in damping inflammation and tissue damage in M. tuberculosis infection. The Journal of Immunology, 2007, 179: 3119–3125.

The intracellular pathogen Mycobacterium tuberculosis infects primarily macrophages. Several different macrophage receptors mediate M. tuberculosis binding and uptake (1). Mycobacterial products, such as the glycolipids phosphatidylinositol mannoside, lipomannan, lipoarabinomannan, lipoproteins, and other mycobacterial factors, may contribute to continued macrophage and dendritic cell (DC)4 activation through pathogen pattern recognition receptors such as TLRs and others. So far, TLR2, TLR4, and TLR1/TLR6 that heterodimerize with TLR2, have been implicated in the recognition of mycobacterial Ags (2, 3), leading to MyD88-dependent activation of proinflammatory and antibacterial effector pathways, which include production of proinflammatory cytokines such as TNF, IL-1, and IL-12, chemokines, and NO (4).

In general, production of these proinflammatory cytokines and chemokines is essential for the recruitment of inflammatory cells at the site of infections and the formation and maintenance of granuloma, which serves to localize and contain not only the bacteria but, also, the inflammatory response to the bacteria. Thus, rigorous control of the inflammatory response is necessary to prevent immunopathology (5). In general, although the immune system is designed to be protective, if left unchecked, excessive or inappropriate activation of immune cells and/or production of proinflammatory cytokines may lead to severe inflammatory diseases. Although the positive functions of TLR and cytokine receptors in inflammation have been widely studied, less is known on how these pathways are negatively regulated (6, 7).

Toll/IL-1R (TIR)8, also known as single Ig IL-1-related receptor (SIGIRR) (8), is a member of the IL-1R family with unique properties. It is structurally characterized by a single extracellular Ig domain, an intracellular TIR domain, and a 95-aa cytoplasmic tail (8, 9). TIR8 has a distinct pattern of expression that includes epithelial tissues and immature DCs. Ligands for TIR8/SIGIRR have not been identified, and searches for accessory functions in signaling complexes have yielded negative results (8). Recently, TIR8 was shown to inhibit NF-κB activation by members of the IL-1/TLR family (9, 10). The inhibitory activity of TIR8 was associated with trapping of TNFR-associated factor 6 and IL-1R-associated kinase 1 (10–12). SIGIRR/Tir8-deficient mice are more susceptible to the systemic toxicity of bacterial LPS (10, 11), intestinal inflammation by dextran sulfate sodium (11), and colitis-associated cancer (13, 14). In vitro, Tir8-deficient DCs, but not macrophages, show increased responsiveness to LPS and CpG oligodeoxynucleotides in terms of production of cytokines and chemokines. Therefore, the finding that Tir8-deficient DCs show increased responsiveness to TLR agonists is consistent with its pattern of expression and its proposed function as a negative regulator of IL-1R/TLR signaling and thus may play a crucial role in damping inflammation.
However, although these reported results have been obtained using LPS or dextran sulfate sodium to induce inflammation and pathology, there is no evidence of such a negative regulatory role for TIR8 during inflammatory responses caused by microorganisms.

We report that TIR8-deficient mice succumb rapidly to infection with *M. tuberculosis*, despite controlling efficiently the infection in different organs. Exaggerated mortality was due to massive liver necrosis and was reversed by in vivo treatment with Abs to IL-1 and TNF-α. Thus, TIR8 plays a key role in damping inflammation and tissue damage in *M. tuberculosis* infection.

**Materials and Methods**

**Mice**

Generation of TIR8-deficient (TIR8<sup>−/−</sup>) mice has been described elsewhere (11). Mice were routinely genotyped by PCR with two primer sets that detected the wild-type and targeted allele. Phenotypic analysis was performed in a 129Sv and C57BL/6 mixed genetic background. TIR8<sup>−/−</sup> mice were littermates of TIR8<sup>−/−</sup> mice. TIR8<sup>−/−</sup> male and female mice (8–14 wk-old) were used in all experiments and each experimental group consisted of five to eight mice. Mice were bred in specific pathogen-free facilities.

**Chemical and reagents**

All chemicals were purchased from Sigma-Aldrich unless otherwise noted. Middlebrook 7H9 liquid medium and 7H10 agar were obtained from Difco Laboratories. Abs used in flow cytometry were obtained from BD Pharmingen or R&D Systems.

**Mycobacteria and infection of mice**

Mice were infected via the intranasal route with *M. tuberculosis* H37Rv (2 × 10<sup>6</sup> CFU in 20 μl). This resulted in reproducible delivery of 50–100 viable CFU of *M. tuberculosis* as confirmed by CFU determination on the lungs of two to three infected mice 1 day postinfection (data not shown).

The tissue bacillary load was quantified by plating serial dilutions of the lung, liver, and spleen homogenates into 7H10 agar as previously described (15).

**Preparation of lung cells and flow cytometric analysis**

To determine cellular infiltrate in the lung, lungs were removed at different weeks after infection and digested in the presence of collagenase to dissociate. A single-cell suspension of the lungs or mediastinal lymph node was prepared by pushing the tissue through a cell strainer. In some experiments, lung mononuclear cells were enriched in T cells by passage through a nylon wool column and then CD4 and CD8 T cells were sorted by anti-CD4 or anti-CD8 immunomagnetic beads (Miltenyi Biotec), following the manufacturer’s instructions (10). The cells were incubated for 24 h at 37°C in complete medium to allow cells and beads to dissociate.

Single-cell suspensions were counted. The samples were triple-stained with fluorochrome-conjugated anti-CD3, anti-CD4, anti-CD8, anti-CD44, anti-CD62L, anti-CD25, anti-pan γδ, anti-Gr1, anti-CD11c, anti-F4/80, and anti-NK1.1 in FACS buffer (0.1% sodium azide, 0.1% BSA, and 20% mouse serum). Purified, PE, FITC, CyChrome, or allophycocyanin conjugated isotype control IgGs were used as control. Following washes, the cells were fixed in 4% paraformaldehyde for 1 h and collected on a FACSCalibur (BD Biosciences). Analysis was performed on CellQuest software (BD Pharmingen).

**Generation of DCs**

Mouse DCs were generated from pooled CD34<sup>+</sup> bone marrow cells using GM-CSF (40 ng/ml) and Flt3 ligand (100 ng/ml). On day 7, DCs were plated at 1 × 10<sup>6</sup>/ml in 0.5 ml (RPMI 1640 plus 1% FCS) and on day 8, cells were stimulated with LPS (1–100 ng/ml), bacillus Calmette-Guérin (BCG) (1:1 and 5:1, multiplicity of infection (MOI)), and *M. tuberculosis* (1:1 and 5:1 MOI). At 24 and 48 h later, supernatants were collected and cytokine content evaluated by commercially available ELISA.

**ELISPOT analysis for IFN-γ**

The ELISPOT method (16) was used to detect IFN-γ secretion by individual CD4 or CD8 T cells from infected mice, after stimulation with BCG-infected DCs in vitro prepared as described (17). In brief, ELISPOT plates (BD Biosciences) were coated with IFN-γ capture Ab overnight at 4°C. The capture Ab was discarded, and the plates were washed and blocked with complete medium for 2 h at room temperature. Purified CD4 or CD8 T cells (150,000/well) were cultured with irradiated BCG-infected wild-type DCs 36–40 h at 37°C. The cells were discarded, and plates were washed with deionized water and PBS/Tween 20. Secondary biotinylated Ab was added for 2 h and incubated at room temperature followed by washing with PBS/Tween 20. Streptavidin-alkaline phosphatase was added to the plates for 1 h followed by washing and development of a color reaction using the substrate 3-amin-o-9-ethylcarbazole substrate reagent kit (BD Biosciences). The reaction was stopped when the spots developed by running the plate under water. The spots were enumerated using an ELISPOT reader.

**ELISA for cytokines**

The concentrations of cytokines (IL-1α, IL-1β, IL-6, TNF-α, and IFN-γ) and chemokines (CCL2, CCL3, and KC) were quantitated by ELISA in lung cell culture supernatants or in serum, using commercially available kits (R&D Systems). Results are reported as mean ± SD of duplicate or triplicate samples.

**Serum transaminases**

Serum alanine aminotransferase (EC 2.6.1.2) and aspartate aminotransferase (EC 2.6.1.1) were measured by the standard photometric method using an Hitachi type 7350 automatic analyzer (Hitachi Chemical Diagnostics) and a commercial kit (Sigma-Aldrich) adapted to small sample volumes, according to (18). Normal range of alanine and aspartate aminotransferase was obtained testing sera from 10 mice, age- and sex-matched with animals used in experimental groups (18).

**Neutralization of cytokines in vivo**

To block inflammatory cytokines, mice were treated with a mixture of neutralizing Abs to IL-1β (hamster IgG1; BD Pharmingen), and TNF-α

**FIGURE 1.** *M. tuberculosis* infection in TIR8<sup>−/−</sup> mice. A, Survival curve of TIR8<sup>+/+</sup> (■) and TIR8<sup>−/−</sup> (□) mice (n = 10) infected intranasally with 2 × 10<sup>4</sup> CFU *M. tuberculosis*. B, The course of *M. tuberculosis* infection in TIR8<sup>+/+</sup> (■) and TIR8<sup>−/−</sup> (□) mice infected intranasally with 2 × 10<sup>4</sup> CFU *M. tuberculosis* and followed against time in lungs, liver, and spleen. Data shown are the mean bacterial counts ± S.D. C, Lung sections from TIR8<sup>+/+</sup> and TIR8<sup>−/−</sup> mice at 4 wk after infection with *M. tuberculosis*. H&E stain at a magnification of ×40.
(rat IgG1; BD Pharmingen), or a mixture of hamster IgG1 and rat IgG1 as a control, starting 2 wk after M. tuberculosis infection and again every week, according to previous details (19). Mice were given i.p. injections of 200 μl of the mixture in sterile PBS, which is equivalent to 100 μg of each Ab.

**Histology**

Tissue samples for histological studies were fixed in 10% normal buffered formalin, followed by paraffin embedment. Sections (5–6 μm) were stained with H&E.

**Statistics**

The double-tailed Student’s t test was used to compare the significance of differences between groups. A value of p < 0.05 was considered significant.

**Results**

M. tuberculosis infection in Tir8−/− mice

To assess the function of TIR8 in the control of M. tuberculosis infection, Tir8+/+ and Tir8−/− mice were infected via the intranasal route with 2000 CFU. This dose did not cause mortality in Tir8+/+ mice over a 16-wk period (Fig. 1A). However, M. tuberculosis infection in Tir8−/− mice resulted in increased mortality, with 60% of mice succumbing by week 6 and 100% of mice succumbing at week 8, at which time experiments were terminated (Fig. 1A).

Despite the exaggerated susceptibility of Tir8−/− mice to M. tuberculosis infection, there was no difference between the two groups of mice in bacterial loads, as determined by CFU counts in the lung, liver, and spleen at 2 and 4 wk postinfection (Fig. 1B), as well as in the few surviving Tir8−/− mice at 6 wk postinfection (data not shown).

We then examined the effect of Tir8 deficiency on the cellular inflammatory response in the lungs, by histology. Whereas in Tir8+/+ mice a moderate cellular infiltration was seen at 4 wk, infection induced extensive cellular recruitment into the lungs of Tir8−/− mice at 4 wk postinfection (Fig. 1C). Thus, Tir8 deficiency led to an overwhelming inflammatory response, which did not influence the extent of the control of the growth of the infecting mycobacteria.

![FIGURE 2. Cytokine and chemokine production by bone marrow-derived DC from Tir8+/+ (■) and Tir8−/− (□) mice. DCs were generated from pooled CD34+ bone marrow cells as described in Materials and Methods. DCs were stimulated with BCG (MOI of 1:1 and 5:1). At 48 h later, supernatants were collected and cytokine and chemokine content evaluated by commercially available ELISA. Results are mean ± SD. *, p < 0.001 and **, p < 0.02.](image)

![FIGURE 3. Lung leukocyte analysis after infection with M. tuberculosis. Lung leukocytes from Tir8+/+ (■) and Tir8−/− (□) mice were isolated and analyzed by flow cytometry at different times after infection. The cells were stained with fluorescent Abs to F4/80 (macrophages), CD11c (bright, DCs), Gr-1 (neutrophils), CD4, CD8, and NK1.1 (NK cells). Results shown are the mean cell numbers ± SD for each cell type. *, p < 0.001 and **, p < 0.01.](image)
Increased cytokine production by Tir8−/− DCs infected with M. tuberculosis

TIR8 is expressed by immature DCs and it has been reported (11) that TIR8-deficient DCs, but not macrophages, show increased responsiveness to LPS and CpG oligodeoxynucleotides in terms of production of cytokines and chemokines. We therefore tested whether similar increased cytokine production occurred upon infection of DCs with BCG or M. tuberculosis. Fig. 2 shows that Tir8−/− DCs infected in vitro with BCG produced significantly higher amounts of cytokines (IL-1β, TNF-α, and IL-6) and chemokines (CCL2, CCL3, and KC), as compared with Tir8+/+ DCs. Similar results were obtained when Tir8−/− DCs were infected in vitro with M. tuberculosis (data not shown).

Cell migration to the lungs during M. tuberculosis infection in Tir8−/− mice

We examined the cellular infiltrate in the lungs of Tir8−/− mice following infection. At predetermined time points, the lungs were removed, the total number of cells was calculated, and flow cytometric analysis was performed to determine cell populations.

The number of DCs (F4/80− CD11c+) in the lungs was not significantly different between Tir8−/− and Tir8+/+ mice. The number of neutrophils and macrophages was significantly greater in Tir8−/− mice than in Tir8+/+ mice at 2 and 4 wk postinfection. The number of CD4, CD8, and NK lymphocytes were not significantly different between Tir8−/− and Tir8+/+ mice, at any time point after infection (Fig. 3).

Cytokine production in the lungs of Tir8−/− mice

Control of M. tuberculosis infection depends on the activation of macrophages by IFN-γ leading to the production of proinflammatory cytokines, such as TNF-α and IL-1β and reactive nitrogen intermediates, such as NO, and the formation and maintenance of the granulomas (20, 21).

Because our results indicated that Tir8−/− mice have more neutrophils and macrophages migrating to the lungs in response to M. tuberculosis infection, ELISA was performed on total lung mono-

nuclear cells to determine whether these components of the antimiycobacterial immune response were affected by Tir8 deficiency. Production of both TNF-α and IL-1β was evident at week 4 postinfection, but Tir8−/− mice had significantly higher production of these cytokines. Conversely, lung mononuclear cells from Tir8+/+ and Tir8−/− mice produced similar amounts of IFN-γ (Table I). The IFN-γ ELISA results were confirmed by analysis of IFN-γ-producing lung CD4 and CD8 T cells, as determined by ELISPOT (see Fig. 4), showing no significant difference between Tir8+/+ and Tir8−/− mice, at all tested time points.

Extensive liver necrosis in M. tuberculosis-infected Tir8−/− mice

These results demonstrate that Tir8−/− mice have more neutrophils and macrophages migrating to the lungs in response to M. tuberculosis infection, with increased production of IL-1β and TNF-α, but normal IFN-γ response by both CD4 and CD8 T cells. Moreover, Tir8−/− mice control the growth of M. tuberculosis as well as Tir8+/+, yet they succumb rapidly after infection. As the globally limited extent of the lung inflammatory pathology does not explain the elevated mortality in Tir8−/− mice, we performed extensive histology analysis of various organs of M. tuberculosis-infected mice.

We found that at 4 wk after infection with M. tuberculosis Tir8−/− mice suffered a massive liver necrosis that involved most of the parenchyma (Fig. 5). Neither M. tuberculosis-infected Tir8+/+ mice nor uninfected Tir8−/− mice had any sign of liver damage or pathology. In agreement with the histology results, serum transaminases were abnormally increased in M. tuberculosis-infected Tir8−/− mice (Table II). Also, the serum levels of IL-1β

Table I. Cytokine concentration in lungs of Tir8+/+ and Tir8−/− mice 4 wk after M. tuberculosis infection

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Tir8+/+ (ng/ml)</th>
<th>Tir8−/− (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0.62 ± 0.24</td>
<td>0.56 ± 0.12</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.56 ± 0.12</td>
<td>0.72 ± 0.24</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>5.40 ± 0.85*</td>
<td>2.90 ± 0.56*</td>
</tr>
</tbody>
</table>

* Tir8+/+ and Tir8−/− mice were infected intranasally with M. tuberculosis and lungs were removed 4 wk later. Leukocyte suspensions were prepared and cultured in the absence of any stimulation for 48 h at 37°C. Supernatants were then collected, and cytokine levels measured by ELISA. Shown are the mean values ± SD; *, p < 0.001.

Table II. Cytokine and transaminases in serum of Tir8+/+ and Tir8−/− mice 4 wk after M. tuberculosis infection

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Tir8+/+ (ng/ml)</th>
<th>Tir8−/− (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0.36 ± 0.12</td>
<td>14.8 ± 3.5*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.44 ± 0.12</td>
<td>8.3 ± 1.2*</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.21 ± 0.1</td>
<td>0.15 ± 0.1</td>
</tr>
<tr>
<td>ALT</td>
<td>34.3 ± 8.2</td>
<td>874.6 ± 26.1*</td>
</tr>
<tr>
<td>AST</td>
<td>48 ± 12.6</td>
<td>1322 ± 128*</td>
</tr>
</tbody>
</table>

* Tir8+/+ and Tir8−/− mice were infected intranasally with M. tuberculosis. Sera were then collected 4 wk later, and cytokine and transaminases levels measured as described in Materials and Methods. Shown are the mean values ± SD; *, p < 0.001.

The number of CD4, CD8, and NK lymphocytes were not significantly different between Tir8+/+ and Tir8−/− mice, at any time point after infection (Fig. 3).

FIGURE 4. IFN-γ produced by T cells in the lungs after infection with M. tuberculosis. Lung CD4 and CD8 T cells were isolated from Tir8+/+ (■) and Tir8−/− (□) mice at the indicated time points after infection and were restimulated in vitro with irradiated BCG-infected wild-type DCs for 36–40 h at 37°C. The number of IFN-γ-producing CD4 and CD8 T cells was quantitated by flow cytometry. The mean ± SD of IFN-γ+ CD4 and CD8 T cells at each time point is shown.

FIGURE 5. Histological image of liver tissues from Tir8+/+ and Tir8−/− mice at 4 wk after infection with M. tuberculosis. Note the liver tissue of Tir8−/− mice, showing serious degeneration of hepatocytes. H&E stain at a magnification of ×40.
and TNF-α were consistently increased (40- and 20-fold, respectively) when compared with *M. tuberculosis*-infected *Tir8*+/+ mice (Table II).

**Neutralization of IL-1β and TNF-α in vivo reverses mortality in *Tir8*−/− mice**

These results clearly show that *M. tuberculosis* infection in the absence of the TIR8 receptor is characterized by an exaggerated inflammatory response with highly elevated systemic levels of IL-1β and TNF-α, leading to massive liver necrosis and increased mortality, despite the successful control of bacterial growth.

To investigate whether the increased production of IL-1β and TNF-α observed in *Tir8*−/− mice was involved in the pathogenesis of tissue damage, we attempted to block inflammatory cytokines in vivo. For this purpose, mice were treated with a mixture of Abs to IL-1β and TNF-α, as described in Materials and Methods. On the same days, control mice received i.p. injections of an equivalent amount of irrelevant Abs. Mice were injected weekly, starting from the third week after infection with *M. tuberculosis* and were scored for mortality. As expected, *Tir8*−/− mice that were left untreated or were treated with control Abs all succumbed by week 8 after infection with *M. tuberculosis* (Fig. 6), whereas *M. tuberculosis*-infected *Tir8*+/+ mice survived until week 16 after infection. Treatment of *Tir8*−/− mice with neutralizing Abs to IL-1β and TNF-α significantly prolonged survival, with only 20% of mice succumbing at week 8 (at which time point 100% mortality was scored in untreated *Tir8*−/− mice) and 60% of mice succumbing at week 14 (Fig. 6). These results therefore indicate that in the absence of the Tir8 receptor, an exaggerated systemic inflammatory response develops to *M. tuberculosis* infection, with elevated levels of IL-1β and TNF-α that cause liver necrosis and mortality.

**Discussion**

Better understanding of the immunological mechanisms of pathogenesis and protection are of essential importance for the design of novel vaccines and immunotherapy against tuberculosis. It has been proposed, that the protective response to *M. tuberculosis* infection requires CD4 and CD8 lymphocytes, the Th1-type cytokines IFN-γ and TNF-α, and activated macrophages (21, 22). The cooperation between the cells and cytokines requires close interaction, which is achieved following migration and granuloma formation in the lungs. The hallmark of infection in the lung is granuloma formation, consisting of clusters of macrophages, lymphocytes and DCs, which physically contains the mycobacteria and creates a microenvironment for immune cell interaction, limiting *M. tuberculosis* growth and dissemination.

The cytokine signals that regulate granuloma formation and persistence are poorly understood, although signaling through IL-1R type 1 (23) and TNFRI plays an essential role (24, 25). TNF-α is a highly potent proinflammatory cytokine with a wide range of activities in both inflammatory and immune responses (26). TNF-α (25, 27) is essential for host resistance against infection with *M. tuberculosis* and other mycobacteria. TNF-deficient mice infected by the aerosol route with *M. tuberculosis* develop normal T cell responses to mycobacterial Ags (28), but are profoundly susceptible to the infection, succumbing with extensive necrosis in the lungs and infected organs.

IL-1 is expressed in macrophages during tuberculosis (29), and activated macrophages demonstrate an increased expression of IL-1β (30, 31). Treatment with IL-1R antagonist was associated with enhanced outgrowth of *M. avium* in lungs, which was accompanied by a reduced influx of inflammatory cells in the pulmonary compartment (32). IL-1R−/− mice, in which IL-1 signaling is absent, succumb to *M. tuberculosis* infection associated with enhanced growth of mycobacteria at the site of infection and in distant organs (23, 33) and IL-1αβ double knockout mice (34) have a profound defect in the generation of the early protective immune response to *M. tuberculosis* infection.

However, although the production of IL-1 and TNF-α, as well as other proinflammatory cytokines is designed to be protective, if left unchecked, their excessive or inappropriate production may lead to severe inflammatory diseases (35, 36). Relevant to *M. tuberculosis* infection, production of elevated amounts of TNF-α results in severe inflammation in the lungs and spleen and early death (37) and IL-1–coated beads are capable of inducing large granulomas in lung tissue (38).

Members of the TLR-IL-1R superfamilies are important in recognizing microbial products and eliciting appropriate immune responses, as well as immunopathology, as documented by exaggerated inflammation in *Tir8*−/− mice (10, 11). This suggests that the TIR8 receptor might play a role in controlling excessive immune responses during *M. tuberculosis* infection.

The function of these inflammatory receptors is tightly regulated by inhibitors acting extracellularly and intracellularly (6, 39). TIR8/SIGIRR has emerged as one nonredundant pathway of negative regulation of TLR-IL-R signaling (9–13).

Results reported in this study are consistent with this view in a murine model of *M. tuberculosis* infection through the mucosal (intranasal) route and show that *Tir8*−/− mice have an exaggerated inflammatory response leading to death, despite their ability to efficiently control bacterial load.

Upon i.n. delivery of a low dose *M. tuberculosis*, 60% of *Tir8*−/− mice succumbed by week 6 and 100% of mice succumbed at week 8, despite the successful control of the growth of *M. tuberculosis* bacilli. Mortality in *Tir8*−/− mice coincided with an overwhelming inflammatory response in the lungs, as documented by increased and sustained numbers of neutrophils and macrophages migrating to the lungs in response to *M. tuberculosis* infection, and increased production of IL-1β and TNF-α. However, this exaggerated inflammatory response does not seem to significantly influence the development of a specific immune response, as demonstrated by the findings that there is no significant difference between *Tir8*−/− and *Tir8*+/+ mice in 1) the number of CD4 and CD8 T cells migrating to the lung, 2) the amount of IFN-γ produced by lung mononuclear cells, and 3) the number of lung CD4 and CD8 T lymphocytes primed to produce IFN-γ upon in vitro stimulation by BCG-infected DCs, as confirmed by ELISPOT analysis.

Recent data in other systems have shown that *Tir8*−/− mice are more susceptible to the systemic toxicity of bacterial LPS (10). In another study (11), *Tir8*−/− mice of a different genetic background had normal susceptibility to systemic LPS toxicity but displayed an exaggerated intestinal inflammatory response to dextran sulfate sodium.
ROLE OF THE TIR8 IN M. tuberculosis INFECTION

The finding that Tir8<sup>−/−</sup> mice had more inflammation in their lungs is intriguing and convincingly supports an immunoregulatory role for this receptor. However, the degree of the inflammatory response in the lungs and the finding that Tir8<sup>−/−</sup> mice control as efficiently as Tir8<sup>+/+</sup> mice M. tuberculosis growth in different organs, does not explain their fast and elevated mortality.

Rather we found that at 4 wk after infection with M. tuberculosis Tir8<sup>−/−</sup> mice suffered an extensive liver necrosis, with abnormally elevated serum transaminases, IL-1β, and TNF-α levels.

In vitro studies confirmed the increased IL-1β, TNF-α, IL-6, and chemokine production by Tir8<sup>−/−</sup> DCs that had been infected with M. tuberculosis: this finding, together with the reported observation that Tir8 is expressed by immature DCs (11), thus point has little direct damage on liver, its elevation could stimulate many cells and stimulates a number of proinflammatory mediators inflammatory response in the lungs and the finding that regulatory role for this receptor. However, the degree of the inflammation in mice deficient in TIR8, an inhibitory member of the IL-1 receptor family. Proc. Natl. Acad. Sci. USA 101: 3522–3526.


15. van der Laarse, V. A. J. Myers, A. C. Scanga, and J. L. Flynn. 2003. CD4<sup>+</sup>, but not CD4<sup>+</sup>IL<sup>−</sup> cells are required for the optimal priming of T cells and control of aerosol M. tuberculosis infection. Immunity 19: 823–825.


