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Interleukin-1 Signaling Is Essential for Host Defense during Murine Pulmonary Tuberculosis

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Interleukin (IL)–1 signaling is required for the containment of infections with intracellular microorganisms, such as *Listeria monocytogenes* and *Leishmania major*. To determine the role of IL-1 in the host response to tuberculosis, we infected IL-1 type I receptor–deficient (IL-1R<sup>−/−</sup>) mice, in which IL-1 does not exert effects, with *Mycobacterium tuberculosis*. IL-1R<sup>−/−</sup> mice were more susceptible to pulmonary tuberculosis, as reflected by an increased mortality and an enhanced mycobacterial outgrowth in lungs and distant organs, which was associated with defective granuloma formation, containing fewer macrophages and fewer lymphocytes, whereas granulocytes were abundant. Lymphocytes were predominantly confined to perivascular areas, suggesting a defective migration of cells into inflamed tissue in the absence of IL-1 signaling. Impaired host defense in IL-1R<sup>−/−</sup> mice was further characterized by a decrease in the ability of splenocytes to produce interferon-γ. Analysis of these data suggests that IL-1 plays an important role in the immune response to *M. tuberculosis*.

Interleukin (IL)–1 is a potent proinflammatory cytokine implicated in numerous physiological processes and in the pathogenesis of a number of inflammatory diseases [1]. IL-1 can bind 2 receptors. Whereas the IL-1R type II is a decoy receptor, binding of IL-1 to IL-1R type I results in signal transduction. Also, IL-1R type I receptor–deficient (IL-1R<sup>−/−</sup>) mice do not respond to IL-1 [2]. Therefore, IL-1R type I seems responsible for the biological actions of IL-1.

The acquired immune response to *Mycobacterium tuberculosis* infection is characterized by the formation of granulomas that consist of macrophages, T cells, and granulocytes. The essential role of an intact Th1 response in host defense against tuberculosis (TB) is illustrated by reports of a markedly enhanced susceptibility to TB of mice deficient for interferon (IFN)–γ [3, 4] or IL-12 [5]. A Th1 response is also protective during other intracellular infections, such as listeriosis and leishmaniasis [6]. IL-1 has been found to play a role in directing the T cell response during these infections. Indeed, both IL-1R<sup>−/−</sup> mice and mice treated with a blocking antibody to IL-1R type I have a greatly decreased ability to control infection with *Listeria monocytogenes* [2, 7]. In addition, IL-1R<sup>−/−</sup> mice displayed higher parasite burdens than normal wild-type mice in a model of cutaneous leishmaniasis, which was associated with an enhanced Th2 response [8]. This suggests that IL-1 is involved in the regulation of Th1/Th2 immune responses to infection with intracellular pathogens.

IL-1 is produced at the site of infection during TB, as shown by elevated levels of IL-1 in bronchoalveolar lavage fluid [9] and IL-1β expression in granulomas in lungs of patients with TB [10]. Also, bronchoalveolar lavage cells obtained from the infected lung of TB patients spontaneously release IL-1β [11], and a relative imbalance in the secretion of IL-1β and its natural inhibitor IL-1 receptor antagonist in favor of the former has been found in patients with active pulmonary TB and a large cavity [12]. This suggests that IL-1 plays a role in host defense to TB in the pulmonary compartment. Indeed, IL-1–coated beads are capable of inducing large granulomas in lung tissue, indicating that IL-1 is essential for the formation and maintenance of granulomas [13]. Furthermore, a possible role for IL-1 in the pathogenesis of TB is suggested by genetic studies that demonstrate that polymorphisms in the IL-1 gene cluster may influence disease expression in human TB [14, 15]. In this study, we determined the role of endogenous IL-1 in the pathogenesis of TB by comparing the course of this disease in IL-1R<sup>−/−</sup> and IL-1 type 1 receptor–present (IL-1R<sup>+/+</sup>) mice intranasally infected with *M. tuberculosis*.

Materials and Methods

Mouse strains. IL-1R<sup>−/−</sup> mice back-crossed 6 times to a C57Bl/6 mouse background (kindly provided by Immunex, Seattle, WA) and...
their normal C57Bl/6 wild-type mice (IL-1R−/−; Harlan Sprague Dawley, Horst, Netherlands) were used. Sex- and age-matched (7- to 8-week-old mice) were used in all experiments. Each experimental group consisted of 6-8 mice studied per time. IL-1R−/− mice were normal in size, weight, and fertility and displayed no abnormalities in leukocyte subsets [16].

Experimental infection. A virulent laboratory strain of M. tuberculosis (H37Rv) was grown in liquid Dubos medium containing 0.01% Tween 80 for 4 days. A replicate culture was incubated at 37°C and stirred gently, harvested at midlog phase, and stored in aliquots at −70°C. Before each experiment, a vial was thawed and washed twice with sterile saline, to clear the mycobacteria of medium. We anesthetized the mice with isoflurane delivered by inhalation. During this brief anesthesia, intranasal inoculation was conducted by placing 105 viable M. tuberculosis organisms in 50-μL NaCl on the nares. The inoculum was placed on plates immediately after inoculation to determine viable counts. Control mice received 50-μL NaCl. After 2 and 5 weeks, mice were anesthetized by FFM (fentanyl citrate, 0.079 mg/mL; fluanisone, 2.5 mg/mL; and midazolam, 1.25 mg/mL) in water; 7.0 mL/kg of this mixture was delivered intraperitoneally) and were killed by bleeding the vena cava inferior.

Reverse transcriptase-polymerase chain reaction (RT-PCR) for cytokine message. Lung tissue samples were homogenized in 1 mL of TRizol Reagent (GibcoBRL, Life Technologies, Grand Island, NY), and total RNA was isolated with chloroform extraction and isopropanol precipitation. The RNA was dissolved in diethyl pyrocarbonate–treated water and quantified by spectrophotometry. cDNA was synthesized by mixing 2-μg RNA with 0.5-μg oligo(dT) (GibcoBRL) and by incubating the solution (12-μL total volume) for 10 min at 72°C. Subsequently, 8 μL of a solution containing 5× first-strand buffer (GibcoBRL) and the Superscript Preamplification system (GibcoBRL) were added, and the final solution was incubated for 60 min at 37°C. For RT-PCR, equivalent amounts of cDNA (5 μL) were amplified with a solution (20 μL) containing 4% dimethyl sulfoxide (Merck, Germany), 12.5-μg bovine serum albumin (Biolabs, Carle Place, NY), 1.25 mM of each dNTPs, 10 μL of PCR buffer (0.67 M Tris, 45 mM NaCl, 0.01% Tween 80, 1 Leu, 12.5-μL PCR buffer (0.67 M Tris-HCl [pH 8.8], 67 mM MgCl2, 0.1 M β-mercaptoethanol, 67 μM EDTA, 0.166 M [NH4]SO4), 0.5 U of AmpliTaq DNA polymerase (Perkin Elmer, Branchburg, NJ), and the forward (F) and the reverse (R) primers (100 μM each). The PCR reactions were carried out in the thermocycler Gene Amp PCR System 9700 (Perkin Elmer, Norwalk, CT) using 1 cycle of 5 min at 94°C and a final extension phase at 72°C for 10 min. The sequences and the cycle numbers were as follows: IL-1α (F): 5′-CTCTAGAGGACCATGCTACAGAC-3′; IL-1α (R): 5′-TGAGGATCCAGGGGAAAACACTG-3′ (30 cycles); IL-1β (F): 5′-TCATGGGATGATGATAACCTGCT-3′; IL-1β (R): 5′-CCACATCTTGAAGGAACACGGAT-3′ (30 cycles); β-actin (F): 5′-GCTGAAAGGACTCTCATGTG-3′; β-actin (R): 5′-GCTGCTTGGCACATTAGTGATG-3′ (24 cycles). The PCR products were separated in 1.5% agarose gel containing half-strength TBE (50 mM Tris, 45 mM boric acid, and 0.5 mM EDTA [pH 8.3]) with 0.5-μg/mL ethidium bromide.

Enumeration of mycobacteria. The lungs, liver, and spleen were harvested and homogenized separately in sterile saline with a tissue homogenizer (Biospec Products, Bartlesville, OK). Ten-fold serial dilutions were plated on Middlebrook 7H11 plates containing oleic acid, albumin, dextrose, and catalase enrichment (Difco, Braun- schweig, Germany) and were incubated at 37°C in sealed bags. Colonies were counted after 3 weeks.

Histological analysis. Lung and liver tissue samples were fixed in 10% neutral buffered formalin. After embedding the samples in paraffin, 4-μm sections were stained with hematoxylin-eosin or Zielh-Neelsen stain for acid-fast bacilli. Slides were then coded and were analyzed by a pathologist for cellular infiltrate. Granulomas were defined as collections of ≥10 macrophages and lymphocytes within the peripheral lung parenchyma [17].

Flow cytometry. Cell suspension of lung tissue was obtained with an automated desegration device (Medimachine System; Dako, Glostrup, Denmark) and resuspended in RPMI (Biowhittaker, Verviers, Belgium). The cell suspension was cleared from debris by filtering through a 40-μm filter (Becton Dickinson, San Jose, CA). Leukocytes were recovered after centrifugation at 20,000 g for 5 min and were counted. A total of 2 × 106 cells was resuspended in PBS containing EDTA 100 mM, sodium azide 0.1%, and bovine serum albumin 5% (cPBS) and placed on ice. Triple staining of lymphocytes was obtained by incubation for 1 h with directly labeled antibodies CD8-FITC, CD3-PE, and CD4-Cy (Pharmingen, San Diego, CA). Cells were then washed twice in ice-cold cPBS and were resuspended for flow cytometric analysis (CaliBrite; Becton Dickinson Immunocytometry Systems, San Jose, CA). At least 5000 lymphocytes were counted. Nonspecific staining was controlled for by incubation of cells with the appropriate control antibodies.

Spleen cell stimulation assays. Single-cell suspensions were obtained by crushing spleens through a 40-μm filter (Pharmingen). Lymphocytes were obtained by Ficoll-Hypaque density gradient centrifugation (Ficoll-Paque; Pharmacia Biotech, Uppsala, Sweden) and were washed extensively. Cells were suspended in RPMI medium containing 10% fetal calf serum and 1% antibiotic-antimycotic (GibcoBRL). Round-bottomed plates were coated overnight with anti-CD3 (clone 145.2c11, diluted 1:20) and were washed with sterile PBS. A total of 106 cells was added to each well, diluted with RPMI containing anti-CD28 (1 μg/mL, Pharmingen), and incubated at 37°C for 48 h. IFN-γ and IL-4 were measured in the supernatant by ELISA according to the manufacturer’s instructions (both from R&D Systems, Abingdon, UK). Detection limit for both was 31.2 pg/mL.

Statistical analysis. Differences in bacterial counts were compared with the Mann-Whitney U test. P < .05 was considered statistically significant.

Results

Production of IL-1α and IL-1β in lungs. To investigate whether IL-1α and IL-1β were produced within the pulmonary compartment during TB, RT-PCR was performed on lung homogenates. Messenger RNA of both IL-1α and IL-1β was detectable in lungs of IL-1R−/− and IL-1R−/− mice after infection with M. tuberculosis (data not shown). In addition, IL-1α and IL-1β protein was elevated in lungs of both mouse strains during the course of TB (figure 1).

IL-1R−/− mice are more susceptible to M. tuberculosis infec-
IL-1R−/− mice have enhanced mycobacterial outgrowth. The rate of mycobacterial outgrowth in the lungs of IL-1R−/− mice was increased, compared with that of IL-1R+/+ mice, as reflected by more M. tuberculosis colony-forming units after 2 weeks (P = .005) and 5 weeks (P < .05; figure 3). M. tuberculosis is known to disseminate in mice [18, 19]. We investigated the role of IL-1 signaling in dissemination of TB by enumeration of mycobacteria in distant organs (figure 3). After 2 weeks, there was no difference in the number of mycobacteria in the spleens and livers of IL-1R−/− and IL-1R+/+ mice. However, after 5 weeks, IL-1R−/− mice had a higher bacterial burden in spleen and liver (P < .05 vs. IL-1R+/+ mice for both organs). After 20 weeks, when the survival experiment was terminated, mycobacteria were counted in lungs of the remaining IL-1R−/− (n = 7) and IL-1R+/+ mice (n = 20). Also at this late time, lungs of IL-1R−/− mice contained more mycobacteria than did those of IL-1R+/+ mice (figure 4).

Differential cellular infiltrate and granulomatous formation response in the lungs of infected IL-1R−/− mice. Two weeks after M. tuberculosis infection, IL-1R−/− mice showed an extensive granulomatous response. At the same time, perivascular and peribronchial inflammation predominantly composed of lymphocytes was present. In IL-1R−/− mice, the perivascular and peribronchial lymphocytic infiltrates dominated the picture with less granulomatous reaction (figure 5A and 5B). After 5 weeks, IL-1R+/+ mice displayed a massive and almost diffuse infiltration of macrophages of 50%–80% of the lung parenchyma, with lesser perivascular and peribronchial lymphocytic infiltration (figure 5C). Necrosis was not present. At variance, IL-1R−/− mice presented small areas of necrosis with fibrin deposition, together with an influx of granulocytes. Apoptotic bod-
with enhanced growth of mycobacteria at the site of infection and in distant organs. The difference in mycobacterial load in IL-1R\(^{-/-}\) and IL-1R\(^{+/+}\) mice was especially large at 20 weeks after infection, when the survival studies were terminated. Indeed, at this time, all IL-1R\(^{-/-}\) mice were still alive and displayed a relatively low number of mycobacteria in their lungs, whereas the lungs of the remaining 7 of 20 IL-1R\(^{+/+}\) mice contained several logs more \textit{M. tuberculosis} colony-forming units. Compared with IL-1R\(^{+/+}\) mice, the granulomatous response of IL-1R\(^{-/-}\) mice was less well defined, showing fewer macrophages and lymphocytes. The impaired host response in IL-1R\(^{-/-}\) mice was further characterized by a dominant inflammatory response, with large numbers of granulocytes and the presence of necrosis, whereas the cellular immune response was diminished as reflected by a reduced ability of splenocytes to produce IFN-\(\gamma\). The inadequate protective immune response of mice lacking the type I IL-1 receptor may be due to a defective granulomatous reaction and possibly a reduced Th1 response.

Clinical outcome in TB correlates with the ability to form granulomas, demonstrating the importance of granulomas in the containment of an \textit{M. tuberculosis} infection. We report that in the absence of IL-1 signaling in a murine model of TB, granulomatous changes in the lungs contained fewer lymphocytes and macrophages in IL-1R\(^{-/-}\) mice. Lymphocytes were predominantly confined to perivascular and peribronchial sites. Therefore, migration of lymphocytes and mononuclear phagocytes from the perivascular region into the inflamed lung seems to be impaired in the absence of IL-1. Therefore, analysis of these data suggests that endogenous IL-1 is important for the recruitment of inflammatory cells into granulomas. In accordance with this hypothesis is the previous finding that recombinant IL-1 can directly induce granuloma formation in lungs [13]. In addition, IL-1 is expressed in macrophages during TB [10], and activated macrophages demonstrate an increased expression of IL-1\(\beta\) [20, 21]. Furthermore, the kinetics of IL-1\(\alpha\) production in lungs during murine TB also is suggestive for a role of this cytokine in the constitution of granulomas [22], and treatment with IL-1 receptor antagonist was associated with enhanced outgrowth of \textit{M. avium} in lungs, which was accompanied by a reduced influx of inflammatory cells in the pulmonary compartment [23]. Interestingly, a lack in lymphocyte migration was also seen in TNF\(^{-/-}\) mice with TB [17].

A protective immune response during TB is initiated by the production of Th1 cytokines from CD4\(^+\) T cells in response to mycobacterial antigens [24]. Indeed, IFN-\(\gamma\)\(^{-/-}\) and IL-12 \(^{-/-}\)
mice are highly susceptible to TB [3–5], suggesting a key role for these cytokines in host defense to TB. Whereas resistance to *M. tuberculosis* in mice is characterized by a Th1 response, disease is associated with a Th2 response [25]. IL-1 has been found to be required for the regulation of Th1 and/or Th2 responses [1, 8]. The absence of IL-1 bioactivity resulted in exacerbation of *L. monocytogenes* and *L. major* infection, suggesting a protective role for IL-1 in the host response to intracellular bacteria [7, 8]. Similarly, in this study, the absence of IL-1 signaling was associated with an impaired host response to TB. The ability of splenocytes from IL-1R−/− mice to produce IFN-γ was decreased, compared with that of IL-1R+/+ mice. These results are in accordance with a previous study that reported reduced IFN-γ production by stimulated draining lymph nodes from IL-1R−/− mice immunized with keyhole limpet hemocyanin [8]. It should be noted, however, that in our...
study, anti-CD3 and CD28 was used to stimulate splenocytes ex vivo, which does not provide adequate information about the antigenic specificity of the responding lymphocytes. Taken together with the fact that the Th1 and/or Th2 dichotomy seems less clear in humans than in mice, the exact role of the possible effect of IL-1 on the Th1/Th2 balance during TB remains to be established.

Upon histologic examination, inflammatory aspects were dominant in IL-1R\(^{-/-}\) mice and were characterized by a large collection of granulocytes and the presence of necrosis. Consistently, an antibody to the IL-1 receptor was associated with granulocyte accumulation in vitro and in mice injected with recombinant human IL-1 [26]. Together, the absence of IL-1 signaling seems to result in the accumulation of granulocytes. In addition to granulocytes, both CD4\(^{+}\) and CD8\(^{+}\) T cells were elevated in lung tissue of IL-1R\(^{-/-}\) mice, as measured by FACS analysis. Considering that CD4\(^{+}\) and CD8\(^{+}\) T cells are involved in protection against \textit{M. tuberculosis} infection [25], analysis of these data indicates that the enhanced recruitment of CD4\(^{+}\) and CD8\(^{+}\) T cells in IL-1R\(^{-/-}\) mice cannot compensate for the reduced resistance of these mice against TB. The exact mechanism by which granulocytes and T cells dominate the lung pathology of IL-1R\(^{-/-}\) mice remain to be determined. Although a highly speculative explanation, the enhanced outgrowth of mycobacteria in IL-1R\(^{-/-}\) mice may result in a relatively increased influx of inflammatory cells in the pulmonary compartment secondary to the absence of negative feedback signals that may be induced on the containment of the infection by a more efficient granulomatous reaction.

In summary, this report demonstrates that IL-1R\(^{-/-}\) mice in which IL-1 signaling is absent are highly susceptible to \textit{M. tuberculosis} infection, with enhanced mycobacterial outgrowth in lungs and distant organs. Loss of resistance to TB was associated with impaired granuloma formation and a lack in Th1-mediated immune response. We conclude that IL-1 has an important role in the generation of an adequate host defense against infection with \textit{M. tuberculosis}.

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References