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Cutting Edge: Caspase-1 Independent IL-1β Production Is Critical for Host Resistance to *Mycobacterium tuberculosis* and Does Not Require TLR Signaling In Vivo

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Abstract

To investigate the respective contributions of TLR versus IL-1R mediated signals in MyD88 dependent control of *Mycobacterium tuberculosis,* we compared the outcome of *M. tuberculosis infection* in MyD88, TRIF/MyD88, IL-1R1, and IL-1β-deficient mice. All four strains displayed acute mortality with highly increased pulmonary bacterial burden suggesting a major role for IL-1β signaling in determining the MyD88 dependent phenotype. Unexpectedly, the infected MyD88 and TRIF/MyD88-deficient mice, rather than being defective in IL-1β expression, displayed increased cytokine levels relative to wild-type animals. Similarly, infected mice deficient in caspase-1 and ASC, which have critical functions in inflammasome-mediated IL-1β maturation, showed unimpaired IL-1β production and importantly, were considerably less susceptible to infection than IL-1β deficient mice. Together our findings reveal a major role for IL-1β in host resistance to *M. tuberculosis* and indicate that during this infection the cytokine can be generated by a mechanism that does not require TLR signaling or caspase-1. *The Journal of Immunology,* 2010, 184: 3326–3330.

Innate resistance to *Mycobacterium tuberculosis* is a key factor in disease outcome as only a small percentage of individuals exposed to this pathogen become actively infected (1). Mice deficient in the TLR/IL-1R family receptor adaptor molecule MyD88 have been shown to be highly susceptible to infection with *M. tuberculosis,* supporting a major role for this pathway

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in innate defense against the bacterium (2, 3). Nevertheless, there has been considerable controversy as to whether the requirement for MyD88 reflects the involvement of a single or multiple TLRs or instead cytokine signaling via IL-1R or IL-18R (4-7). Despite convincing evidence for the importance of TLR/TLR-ligand interactions in the response to *M. tuberculosis* in vitro, a number of studies have indicated that IL-1R1 signaling may play a more dominant role in MyD88-dependent resistance in vivo (8-10).

The two known ligands for IL-1R1 are IL-1α and IL-1β. These cytokines, although both induced by NF-κb dependent pathways, have different posttranslational processing requirements. Expression of the highly proinflammatory cytokine IL-1β is tightly controlled through the combination of two distinct triggers: signal 1 that induces the expression of pro-IL-1β and signal 2 that results in IL-1β protein maturation via caspase-1 (11). Signal 1 typically involves triggering by a TLR ligand and IL-1 itself can serve as positive feedback regulator of this pathway. Signal 2, in contrast, involves a number of poorly defined recognition events that lead to the assembly of a protein complex known as the inflammasome that activates caspase-1, thereby cleaving the proform of IL-1β into mature IL-1β (12). The apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) is a critical component of most inflammasomes.

In this paper, in addition to confirming the major role of IL-1R1 signaling in MyD88-dependent host resistance to *M. tuberculosis,* we have now identified IL-1β as a critical ligand in this pathway. Unexpectedly, our experiments reveal that although IL-1β induction by *M. tuberculosis* in vitro depends on TLR triggering and the inflammasome for signals 1 and 2 respectively, both triggers are dispensable for IL-1β production in vivo in mice infected with the pathogen.

Materials and Methods

Mice

C57BL/6 mice were purchased from Taconic Farms (Germantown, NY). All knockout strains were on a full C57BL6 background or were backcrossed for a minimum of six generations. MyD88$^{-/-}$ mice and IL-18$^{-/-}$ were originally obtained from S. Akira (Osaka University, Osaka, Japan) and IL-1β$^{-/-}$ mice from Y.Iwakura (University of Tokyo,Tokyo, Japan).N6 backcrossed IL-1R1$^{-/-}$ mice were first purchased from The Jackson Laboratory (Bar Harbor, ME). Results from these mice were later confirmed using fully backcrossed IL-1R1$^{-/-}$ mice generously provided by Drs. Dirk Schlueter and Horst Bluethmann (Pharmaceutical Research Gene Technology, F. Hoffmann-La Roche, Basel, Switzerland). TLR2/9$^{-/-}$ and TRIF/MyD88$^{-/-}$ were generously supplied by D. Golenbock and E. Lien (University of Massachusetts, Worcester, MA). The TLR2/9$^{-/-}$ mice were fully backcrossed. ASC$^{-/-}$ mice were originally derived at Millennium Pharmaceuticals, Cambridge, MA (13). Casp1$^{-/-}$ mice were generated as described and subsequently backcrossed to C57BL6 (N6) (14). All animals (8–15 wk old of both sexes) were bred in an AALAC-accredited animal facility at the National Institute of Allergy and Infectious Diseases, National Institutes of Health (Bethesda, MD).

In vivo and in vitro M. tuberculosis *infections*

Low-dose (50–100 CFU/mouse) aerosol infection with the H37Rv strain of *M. tuberculosis* and determination of bacterial growth in tissues was performed as previously described (3, 4). Bone marrow-derived macrophages (BMDMs), were generated as described (3, 4) and exposed to H37Rv at a multiplicity of infection of one (MOI:1) in complete media for 48 h after which the supernatants were harvested for ELISA.

Tissue harvest and ELISA

Bronchoalveolar lavage fluid (BALF) was obtained by washing the lung airways with 1 ml sterile PBS/mouse. Aliquots were taken for bacterial load determination and after centrifugation the cell-free supernatants assayed by ELISA. Lungs were then perfused with 10 ml sterile PBS and mechanically disrupted by passing through a 100-μm cell strainer in a total volume of 4 ml PBS 1% FCS. An aliquot was removed for determining bacterial load. After centrifugation, the cell-free lung suspensions were analyzed by ELISA. IL-1β was measured using ELISA kits from eBioscience (San Diego, CA) and R&D Systems (Minneapolis, MN) with similar results. IL-1α, IFNγ, TNFα, and IL-1R antagonist were determined with R&D DuoSet kits. High-mobility group box 1 (HMGB1) was detected using the HMGB1 ELISA Kit II from IBL Hamburg (Hamburg, Germany). Total nitrite was measured using the nitrate reduction assay from R&D Systems.

Histopathology

Lungs were fixed by inflating the tissues with 4% formaldehyde, sectioned and stained with H&E or by the Ziehl-Neelsen method to visualize acid-fast mycobacteria.

RT-PCR

Total RNA was isolated from lungs, and real-time RT-PCR was performed as previously described (3, 4). The following primer pairs were used: for *hprt: 5′*-GTTGGTTACAGGCCAGACTTTGTTG-3′ (forward) and 5′-GAGGGTAGGCTGGCCTATAGGCT-3′ (reverse); *il-1r1: 5′*-TGTAATGAAAGACGGCACACC (forward) and 5′-TCTTCTTTGGGTATTGCTTGG-3′ (reverse)

Western blotting

Cell-free lung suspensions from 4-wk infected wild-type (WT), IL-1β$^{-/-}$, and casp1$^{-/-}$ mice were loaded in equal volumes and separated on a 12% polyacrylamide NuPAGE gel (Invitrogen, Carlsbad, CA), and the proteins transferred to a Hybond-P PVDF membrane (GE Healthcare, Piscataway, NJ). A goat anti-mouse polyclonal IgG Ab (R&D Systems) was used to detect IL-1β.

Results and Discussion

IL-1β is essential for MyD88-dependent host resistance to M. tuberculosis

To help clarify the respective roles of upstream signals determining MyD88-dependent host resistance to *M. tuberculosis,* we performed an extensive series of experiments directly comparing the susceptibility of seven different mouse strains deficient in specific TLR/IL-1R family receptors, adaptor molecules or cytokines. Confirming published data (3, 8), MyD88$^{-/-}$ and IL-1R1$^{-/-}$ mice succumbed rapidly to low−dose aerosol infection, displaying 2–3 log increases in bacterial loads in lung and BALF (Fig.1*A,* 1*B*) and extensive pulmonary necrosis (Fig.1*C,* 1*D* and data not shown) 4 wk postinfection (p.i). No further increase in susceptibility was evident in mice doubly deficient in both MyD88 and the alternative TLR adaptor TRIF (Fig.1*A*, 1*B*). Also as previously reported, IL-18$^{-/-}$ and TLR2/9$^{-/-}$ mice succumbed to infection earlier than WT animals (Fig.1*A*) (4, 15). However, their increase in susceptibility was minor relative to the acute mortality seen in the MyD88/IL-1R1 deficient strains. Importantly, mice deficient in IL-1β alone recapitulated the phenotype observed in the MyD88$^{-/-}$ mice in terms of survival, bacterial load and pulmonary pathology (Fig.1).

The induction of reactive nitrogen species mediated by IFNγ and TNFγ signaling has been demonstrated to be critical for the control of M. tuberculosis infection in mice (1). Despite their increased susceptibility, infected IL-1β$^{-/-}$ mice expressed even higher levels of these mediators in lungs than WT animals (Fig. 2*A*). In contrast, IL-12/23 p40 expression was reduced in IL-1β$^{-/-}$ lungs (Fig. 2*B*), indicating that the increase in IFNγ is not due to elevated p40 expression and suggesting that defective IL-1β/IL-1R1 signaling may contribute to the previously reported defect in p40 production in MyD88$^{-/-}$ mice (2-4). Importantly, IL-1α levels were markedly elevated in the infected IL-1β$^{-/-}$ animals, arguing that in the absence of IL-1β, IL-1α is not sufficient to mediate IL-1R1 dependent host resistance (Fig. 2*C*). Moreover, HMGB1, which, in common with IL-1α, is released by dyingnecrotic cells (16-18), was significantly increased in the lungs of infected IL-1β$^{-/-}$ mice (Fig. 2*C*). Expression of the endogenous IL-1R antagonist was also highly upregulated in the lungs of both WT and IL-1β$^{-/-}$ mice (Fig. 2*D*), which could explain why in the latter animals IL-1α is not protective.

The above findings support the hypothesis that IL-1R1 rather than TLR or IL-18 signaling underlies the critical role of MyD88 in early host resistance to M. tuberculosis and for the first time assign to IL-1β a major nonredundant function in acute control of this pathogen. Based on our data, the loss in acute bacterial control observed in IL-1β$^{-/-}$ mice is not due to a downstream defect in the induction of the host-protective mediators IFNγ, TNFα, and NO. In addition, infected IL-1 β$^{-/-}$ mice showed no impairment in *M. tuberculosis*-specific CD4 T cell priming measured by MHC class II tetramer staining and IFNγ cytokine production after cognate peptide stimulation (K. Mayer-Barber, D. Barber, and A. Sher, manuscript in preparation). Further information concerning the cellular sources and targets of IL-1β in the lungs of *M. tuberculosis* infected mice and the downstream effects of IL-1β signaling will be important in identifying the specific host-protective pathway involved.

IL-1β expression in vivo does not require TLR signaling

MyD88 could regulate IL-1β/IL-1R1 dependent resistance to *M. tuberculosis* through its downstream role in IL-1R1 signal transduction and/or its function in upstream TLR/NFκB dependent *il1b* gene induction as signal 1 in the expression of the cytokine. To address this point, we compared IL-1β levels 4 wk p.i. in lungs and BALF of the MyD88 and IL-1β/IL-1R1 deficient mouse strains analyzed above. Importantly, we found that IL-1β expression was significantly induced in MyD88$^{-/-}$, TRIF/MyD88$^{-/-}$, and IL-1R1$^{-/-}$ mice at levels that actually exceeded those seen in WT animals (Fig. 3*A*). Consistent with the latter findings, significant induction of IL-1β mRNA was also observed in the lungs of IL-1R1$^{-/-}$ and MyD88$^{-/-}$ mice (Fig. 3*B*). In direct contrast to these in vivo observations documenting MyD88 independent IL-1β synthesis, and consistent with previous work (19), a major defect in IL-1β production measured by the same ELISA assay, was seen when BMDMs from MyD88$^{-/-}$ or TLR2/9$^{-/-}$ mice were stimulated in vitro with M. tuberculosis (Fig. 3*C*, data not shown).

Together, these observations suggest that MyD88 regulates host resistance to *M. tuberculosis* at the level of IL-1β/IL-1R1 signaling rather than TLR-mediated triggering of cytokine production and unexpectedly show that IL-1β can be induced in vivo by an unidentified pathway that requires neither TLR/TRIF/MyD88 signaling nor IL-1R1/MyD88 as a positive feedback loop.

Nevertheless, our findings do not rule out a nonessential role of TLR signaling for IL-1β induction in WT mice, particularly at earlier stages of infection. One scenario is that in the knockout mice proinflammatory/necrotic mediators override the usual role of TLR stimulation in IL-1β expression. One major candidate is TNFα, which we found to be highly expressed in lungs of infected TRIF/MyD88$^{-/-}$, MyD88$^{-/-}$, and IL-1R1$^{-/-}$ mice (data not

shown) and which has recently been demonstrated to induce IL-1β in vitro in the absence of TLR signaling (12, 17).

Caspase-1 and ASC play minimal roles in M. tuberculosis-induced IL-1β production and host resistance to infection

To examine the requirement for caspase-1 and inflammasome-mediated IL-1β expression (i.e., signal 2) in IL-1β mediated host resistance, we assessed the response of mice deficient in ASC and caspase-1 (casp1$^{-/-}$) to *M. tuberculosis* infection. BMDMs from both ASC$^{-/-}$ and casp1$^{-/-}$ mice showed major defects in IL-1β production in response to *M. tuberculosis* stimulation in vitro (Fig. 4*A*). In striking contrast, IL-1β expression was unimpaired in *M. tuberculosis* infected ASC$^{-/-}$ and casp1$^{-/-}$ mice (Fig. 4*B*) in both cell-free lung suspensions and BALF. Consistent with their unexpectedly normal levels of IL-1β, the majority of both ASC$^{-/-}$ and casp1$^{-/-}$ animals survived the acute phase of infection displaying approximate mean survival times of 6 and 3 mo, respectively (Fig. 4*C*). When compared with WT animals, pulmonary bacterial burdens were unchanged in ASC$^{-/-}$ mice and moderately but significantly elevated in casp1$^{-/-}$ animals (Fig. 4*D*). Western blotting of cell-free lung suspension, 4 wk p.i. confirmed that IL-1β is cleaved into its 17 kD mature form in both WT, as well as casp1$^{-/-}$ mice (Fig. 4*E*).

In contrast to findings in other infectious disease models (20-22), our data argue that the production of mature host-protective IL-1β during *M. tuberculosis* infection can occur independently of caspase-1 activation and ASC-containing inflammasomes. This process likely involves either a caspase-1 independent pathway for IL-1β processing in macrophages and dendritic cells, and/or a distinct cellular source, such as neutrophils, which can process IL-1β without a requirement for caspase-1 (12, 23, 24). It should be noted that a partial loss in host resistance was observed in both the casp1$^{-/-}$ and ASC$^{-/-}$ mice. This could reflect a contribution of the inflammasome in the processing of IL-1β and/or IL-18, and/or functions of caspase-1 and ASC distinct from cytokine cleavage acting late in infection (15, 25, 26).

Thus, although our findings establish IL-1β as a critical component in innate resistance to *M. tuberculosis,* they also indicate that the pathways for expression and regulation of the cytokine in *M. tuberculosis* infection are complex and may involve mechanisms that do not fit the classical paradigms of TLR recognition and inflammasome-mediated caspase-1 processing seen in other infections or for that matter in the response to *M. tuberculosis* in in vitro systems.

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Abbreviations used in this paper

BALF	bronchoalveolar lavage fluid
BMDM	bone marrow-derived macrophages
HMGB1	high-mobility group box 1
MOI:1	multiplicity of infection of one

p.i.	postinfection
WT	wild type

References

1. North RJ, Jung YJ. Immunity to tuberculosis. Annu. Rev. Immunol. 2004; 22:599–623.
2. Fremond CM, Yeremeev V, Nicolle DM, Jacobs M, Quesniaux VF, Ryffel B. Fatal Mycobacterium tuberculosis infection despite adaptive immune response in the absence of MyD88. J. Clin. Invest. 2004; 114:1790–1799. [PubMed: 15599404]
3. Scanga CA, Bafica A, Feng CG, Cheever AW, Hieny S, Sher A. MyD88-deficient mice display a profound loss in resistance to Mycobacterium tuberculosis associated with partially impaired Th1 cytokine and nitric oxide synthase 2 expression. Infect. Immun. 2004; 72:2400–2404. [PubMed: 15039368]
4. Bafica A, Scanga CA, Feng CG, Leifer C, Cheever A, Sher A. TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis. J. Exp. Med. 2005; 202:1715–1724. [PubMed: 16365150]
5. Hölscher C, Reiling N, Schaible UE, Hölscher A, Bathmann C, Korbel D, Lenz I, Sonntag T, Kröger S, Akira S, et al. Containment of aerogenic Mycobacterium tuberculosis infection in mice does not require MyD88 adaptor function for TLR2, -4 and -9. Eur. J. Immunol. 2008; 38:680–694. [PubMed: 18266299]
6. Korbel DS, Schneider BE, Schaible UE. Innate immunity in tuberculosis: myths and truth. Microbes Infect. 2008; 10:995–1004. [PubMed: 18762264]
7. Reiling N, Ehlers S, Hölscher C. MyDths and un-TOLLed truths: sensor, instructive and effector immunity to tuberculosis. Immunol. Lett. 2008; 116:15–23. [PubMed: 18191460]
8. Fremond CM, Togbe D, Doz E, Rose S, Vasseur V, Maillet I, Jacobs M, Ryffel B, Quesniaux VF. IL-1 receptor-mediated signal is an essentialcomponent of MyD88-dependent innate response to Mycobacterium tuberculosis infection. J. Immunol. 2007; 179:1178–1189. [PubMed: 17617611]
9. Sugawara I, Yamada H, Hua S, Mizuno S. Role of interleukin (IL)-1 type 1 receptor in mycobacterial infection. Microbiol. Immunol. 2001; 45:743–750. [PubMed: 11791667]
10. Yamada H, Mizumo S, Horai R, Iwakura Y, Sugawara I. Protective role of interleukin-1 in mycobacterial infection in IL-1 alpha/beta double-knockout mice. Lab. Invest. 2000; 80:759–767. [PubMed: 10830786]
11. Mariathasan S, Monack DM. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. Nat. Rev. Immunol. 2007; 7:31–40. [PubMed: 17186029]
12. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annu. Rev. Immunol. 2009; 27:519–550. [PubMed: 19302047]
13. Sutterwala FS, Ogura Y, Szczepanik M, Lara-Tejero M, Lichtenberger GS, Grant EP, Bertin J, Coyle AJ, Galán JE, Askenase PW, Flavell RA. Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. Immunity. 2006; 24:317–327. [PubMed: 16546100]
14. Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS, Flavell RA. Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. Science. 1995; 267:2000–2003. [PubMed: 7535475]
15. Sugawara I, Yamada H, Kaneko H, Mizuno S, Takeda K, Akira S. Role of interleukin-18 (IL-18) in mycobacterial infection in IL-18-gene-disrupted mice. Infect. Immun. 1999; 67:2585–2589. [PubMed: 10225924]
16. Bianchi ME, Manfredi AA. High-mobility group box 1 (HMGB1) protein at the crossroads between innate and adaptive immunity. Immunol. Rev. 2007; 220:35–46. [PubMed: 17979838]
17. Franchi L, Eigenbrod T, N G. Cutting edge: TNF-alpha mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation. J. Immunol. 2009; 183:792–796. [PubMed: 19542372]

18. Kono H, Rock KL. How dying cells alert the immune system to danger. Nat. Rev. Immunol. 2008; 8:279–289. [PubMed: 18340345]

19. Kleinnijenhuis J, Joosten LA, van de Veerdonk FL, Savage N, van Crevel R, Kullberg BJ, van der Ven A, Ottenhoff TH, Dinarello CA, van der Meer JW, Netea MG. Transcriptional and inflammasome-mediated pathways for the induction of IL-1beta production by Mycobacterium tuberculosis. Eur. J. Immunol. 2009; 39:1914–1922. [PubMed: 19544485]

20. Mariathasan S, Weiss DS, Dixit VM, Monack DM. Innate immunity against Francisella tularensis is dependent on the ASC/caspase-1 axis. J. Exp. Med. 2005; 202:1043–1049. [PubMed: 16230474]

21. Ichinohe T, Lee HK, Ogura Y, Flavell R, Iwasaki A. Inflammasome recognition of influenza virus is essential for adaptive immune responses. J. Exp. Med. 2009; 206:79–87. [PubMed: 19139171]

22. Sutterwala FS, Mijares LA, Li L, Ogura Y, Kazmierczak BI, Flavell RA. Immune recognition of Pseudomonas aeruginosa mediated by the IPAF/NLRC4 inflammasome. J. Exp. Med. 2007; 204:3235–3245. [PubMed: 18070936]

23. Greten FR, Arkan MC, Bollrath J, Hsu LC, Goode J, Miething C, Göktuna SI, Neuenhahn M, Fierer J, Paxian S, et al. NF-kappaB is a negative regulator of IL-1beta secretion as revealed by genetic and pharmacological inhibition of IKKbeta. Cell. 2007; 130:918–931. [PubMed: 17803913]

24. Guma M, Ronacher L, Liu-Bryan R, Takai S, Karin M, Corr M. Caspase 1-independent activation of interleukin-1beta in neutrophil-predominant inflammation. Arthritis Rheum. 2009; 60:3642–3650. [PubMed: 19950258]

25. Schneider BE, Korbel D, Hagens K, Koch M, Raupach B, Enders J, Kaufmann SH, Mittrucker HW, Schaible UE. A role of interleukin 18 for protective immunity against Mycobacterium tuberculosis. Eur J Immunol. 2010; 40:396–405. [PubMed: 19950174]

26. Shao W, Yeretssian G, Doiron K, Hussain SN, Saleh M. The caspase-1 digestome identifies the glycolysis pathway as a target during infection and septic shock. J. Biol. Chem. 2007; 282:36321–36329. [PubMed: 17959595]

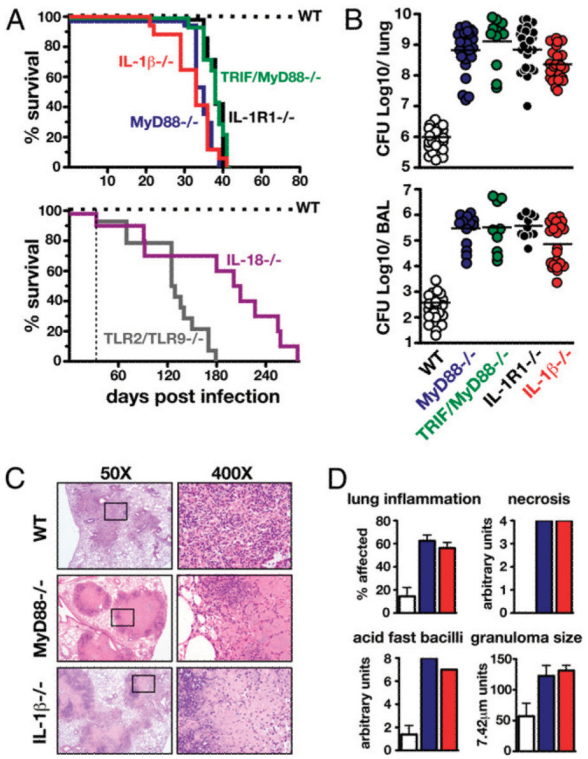

FIGURE 1.

M. tuberculosis infected IL-1β$^{-/-}$ mice display acute mortality and elevated bacterial loads. Survival of TLR2/TLR9$^{-/-}$ (dark gray), TRIF/MyD88$^{-/-}$ (green), MyD88$^{-/-}$ (blue), IL-18$^{-/-}$ (purple), IL-1β$^{-/-}$ (red), IL-1R1$^{-/-}$ (black), and C57BL6 WT (dotted black line, white circle) mice after low dose aerosol exposure to H37Rv. Vertical dotted line in lower panel represents survival of IL-1β$^{-/-}$ mice (*A*). Data are representative of at least two independent experiments each involving 5–12 mice per group. At 4 wk p.i. bacterial loads were measured in BALF and lung suspensions from the strains indicated (*B*). Data are pooled from 3–6 independent experiments. *C,* Representative H&E-stained sections from lungs of C57BL6 WT, MyD88$^{-/-}$ and IL-1β$^{-/-}$ mice are shown at 4 wk p.i. Original magnifications ×50 and ×400. *D,* Presents % lung affected, mean granuloma sizes, as well as the relative number of acid fast bacilli and degree of necrosis scored blindly.

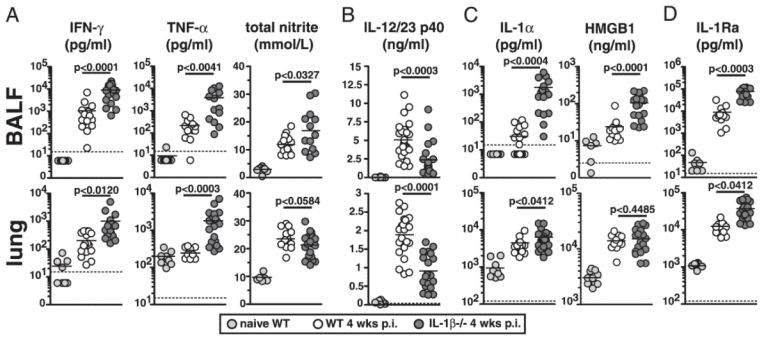

FIGURE 2.

Effect of IL-1β deficiency on cytokine and total nitrite levels in lungs of *M. tuberculosis*-infected mice. Indicated cytokines were measured by ELISA in BALF and cell-free lung suspensions of uninfected C57BL6 WT (light gray) as well as 4 wk infected C57BL6 WT (white) and IL-1$\beta^{-/-}$ (dark gray) mice (*A*–D). Total nitrite was measured in the same samples by Griess assay after nitrate reduction (*A*). Dotted lines indicate limits of detection of the respective ELISA. Statistical comparisons were performed by Student *t* test and *p* values are depicted. Data shown are pooled from at least three independent experiments.

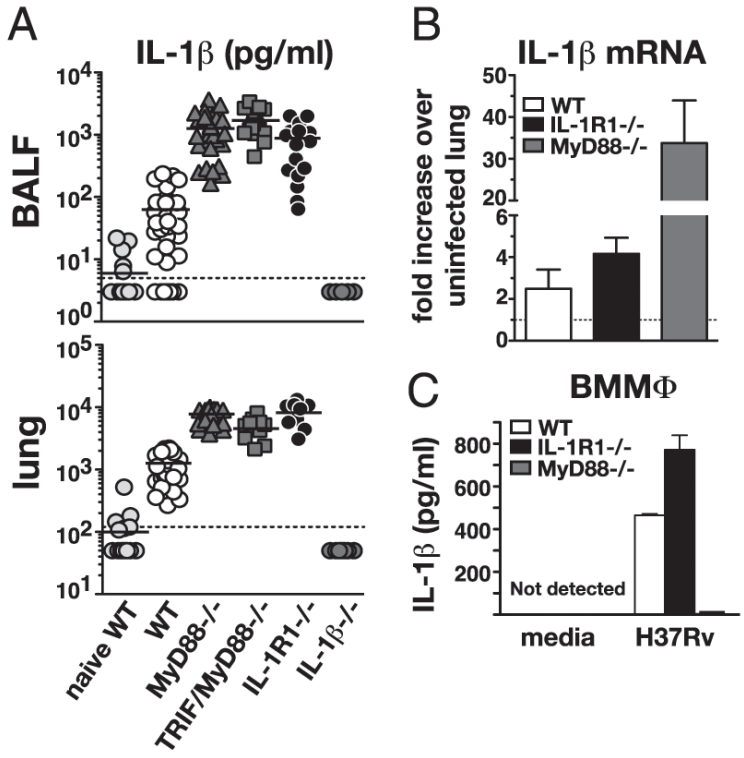

FIGURE 3.

Neither TRIF/MyD88 nor IL-1R1 are required for IL-1β induction in vivo. IL-1β was measured by ELISA in BALF and cell-free lung suspensions of the indicated mouse strains at 4 wk p.i. (*A*). Dotted line indicates limit of detection. Data shown are pooled from at least four independent experiments. IL-1β mRNA levels measured by RT-PCR in lungs of 4 wk infected C57BL6 WT (white), MyD88$^{-/-}$ (dark gray) and IL-1R1$^{-/-}$ (black) mice (*B*). Data shown represent the mean 6 SD-fold increase over uninfected WT controls (dotted line) for two independent experiments with a minimum of five mice per group. IL-1β measured by ELISA in supernatants of BMDM from mouse strains listed (*B*) with and without exposure to live H37Rv (MOI:1) for 48 h (*C*). Data shown are means ± SD and are representative of two independent experiments.

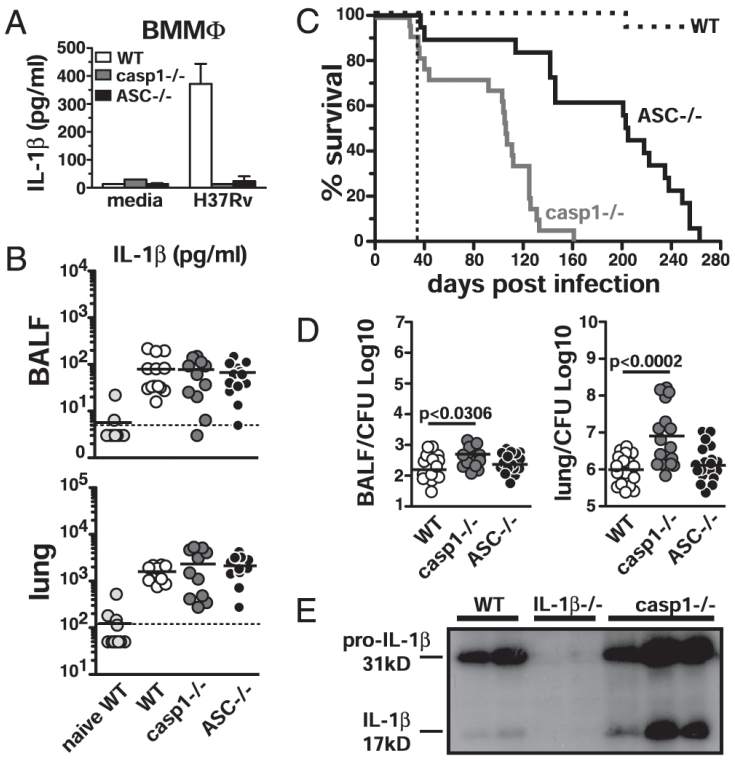

FIGURE 4.

Host resistance and pulmonary IL-1β levels in *M. tuberculosis* infected casp1$^{-/-}$ and ASC$^{-/-}$ mice. IL-1β measured by ELISA in supernatant of BMDM from WT, casp1$^{-/-}$ and ASC$^{-/-}$ mice with and without exposure to live H37Rv (MOI:1) for 48 h (A). Data shown are the means 6 SD and are representative of two independent experiments. B, IL-1β levels in BALF and cell-free lung suspensions of the 4 wk infected mouse strains indicated are depicted. Dotted lines indicate the limit of detection of the ELISA assay. Data shown are pooled from at least three independent experiments. C57BL6 WT, casp1$^{-/-}$ and ASC$^{-/-}$ mice were infected with H37Rv by aerosol exposure and their survival monitored (C). Vertical dotted line represents survival of IL-1β$^{-/-}$ mice. Data are pooled from two independent experiments each with 8-12 mice per group. Bacterial loads in BALF and lung suspension at 4 wks p.i. of the same mouse strains (D). Data are pooled from four independent experiments. E, Western blot detection of IL-1β in cell-free lung suspensions at 4 wk p.i. Each lane represents an individual mouse. In (B) and (D), differences that were statistically significant ($p < 0.05$) are indicated.