Toll-Like Receptor 4-Deficient Mice Have Reduced Bone Destruction following Mixed Anaerobic Infection

LINDA HOU, HAJIME SASAKI, AND PHILIP STASHENKO*

Department of Cytokine Biology, Forsyth Institute, Boston, Massachusetts 02115

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C3H/HeJ mice have an impaired ability to respond to lipopolysaccharide (LPS) due to a mutation in the gene that encodes Toll-like receptor 4 (TLR4). The effect of TLR4 deficiency on host responses to endodontic infections is unknown. In the present study, we compared periapical bone destruction, sepsis, and inflammatory cytokine production in LPS-hyporesponsive C3H/HeJ and wild-type control C3H/HeOuJ mice. The mandibular first molars of both strains were subjected to pulpal exposure and infection with a mixture of four anaerobic pathogens, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Streptococcus intermedius*, and *Peptostreptococcus micros*. At sacrifice on day 21, TLR4-deficient C3H/HeJ mice had significantly reduced periapical bone destruction compared to wild-type C3H/HeOuJ mice ($P < 0.001$). The decreased bone destruction in C3H/HeJ correlated with reduced expression of the bone resorptive cytokines interleukin 1 α (IL-1 α) ($P < 0.01$) and IL-1 β ($P < 0.05$) as well as the proinflammatory cytokine IL-12 ($P < 0.05$). No significant differences were seen in the levels of gamma interferon, tumor necrosis factor alpha (TNF- α), or IL-10 between the two strains. The expression of IL-1 α , IL-1 β , TNF- α , IL-10, and IL-12 were all significantly reduced in vitro in macrophages from both TLR4-deficient C3H/HeJ and C57BL/10ScNcr strains, compared to wild-type controls. Notably, the responses of TLR4-deficient macrophages to both gram-positive and gram-negative bacteria were similarly reduced. Neither C3H/HeJ nor C3H/HeOuJ mice exhibited orofacial abscess development or infection dissemination as determined by splenomegaly or cachexia. We conclude that intact TLR function mediates increased proinflammatory responses and bone destruction in response to mixed anaerobic infections.

Innate recognition of bacterial products constitutes a principal bulwark of host defense against infection. Innate mechanisms, including phagocytic leukocytes and cytokines, play a central role in the pathogenesis of oral infections (5, 17). Strong links have been shown between defects in polymorphonuclear leukocytes and increased periapical and periodontal disease (12, 26). Other responses, particularly the production of proinflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF- α), mediate tissue destruction, including bone resorption (2, 29). Therefore, modulation of innate responses to decrease the expression and activity of inflammatory cytokines represents a potential way of ameliorating alveolar bone destruction.

The recently identified family of Toll-like receptors (TLRs), homologous to *Drosophila Toll*, are key participants in innate recognition of pathogens (16). TLRs are characterized structurally by an extracellular leucine-rich repeat domain and a cytoplasmic domain that is homologous to the signaling domain of the IL-1 receptor (IL-1R). Moreover, the signal transduction pathway for TLRs and IL-1R that leads to cytokine expression is also intertwined with TNF receptor signaling pathways (16). To date, the sequences of seven TLRs have been reported in humans and mice (24). There is evidence that both TLR2 and TLR4 are involved in responses to bacterial lipopolysaccharide (LPS), leading to the expression of proinflammatory cytokines IL-1, TNF- α , IL-6, and IL-8 (10, 14, 19, 43). Recently, TLR2 has also been shown to mediate responses to gram-positive bacterial cell wall components, including peptidoglycan (34) and lipoteichoic acid (27, 44). To date, the role

of TLRs in responses to oral pathogens and in alveolar bone destruction is unknown.

The LPS hyporesponsive mouse strains C3H/HeJ and C57BL/10ScCr have mutations that map to a single autosomal *lps* locus (42). The consequence of this hyporesponsiveness is decreased susceptibility to septic shock (41) and enhanced susceptibility to challenge with some gram-negative pathogens (21). Recently, C3H/HeJ mice were shown to have an inactivating point mutation within the signal transducing domain of the *Tlr4* gene (22), whereas C57BL/10ScCr mice exhibit a deletion of *Tlr4* (23).

In the present study, we compared infection-stimulated intraosseous bone resorption and dentoalveolar abscess formation in TLR4-deficient LPS-hyporesponsive C3H/HeJ and wild-type control C3H/HeOuJ mice. The results demonstrate that TLR4 function significantly enhances inflammatory responses and bone destruction in this model.

MATERIALS AND METHODS

Animals. C3H/HeJ (LPS hyporesponsive) and C3H/HeOuJ (LPS normoresponsive) mouse strains were purchased from Jackson Laboratory, Bar Harbor, Maine. LPS hyporesponsive C57BL/10ScNcr and wild-type control C57BL/10ScSn mouse strains were obtained from Frederick Cancer Research and Developmental Center, National Cancer Institute, Frederick, Md. Animals were maintained in laminar flow isolators in the Forsyth Institute Animal Facility under pathogen-free conditions.

Pulp exposure. C3H/HeJ and C3H/HeOuJ mice ($n = 10$ each), 6 to 8 weeks of age, were anesthetized by the intramuscular injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) in sterile phosphate-buffered saline. The pulps of the mandibular first molars were exposed on day 0 by using a portable, variable-speed electric handpiece (Osada Electric, Los Angeles, Calif.) and a sterile, size 1/4, round bur under a surgical microscope (model MC-M92; Seiler, St. Louis, Mo.). The pulp chambers were opened until the entrances of the canals could be visualized and probed with a no. 10 endodontic file.

Infection with pathogens. Tryptic soy broth with yeast agar plates of four common endodontic pathogens, *Prevotella intermedia* ATCC 25611, *Streptococcus intermedius* ATCC 27335, *Fusobacterium nucleatum* ATCC 25586, and *Peptostreptococcus micros* ATCC 33270, were grown under anaerobic conditions (80% N₂, 10% H₂, and 10% CO₂), were harvested, and were cultured in myco-

* Corresponding author. Mailing address: Department of Cytokine Biology, Forsyth Institute, 140 The Fenway, Boston, MA 02115. Phone: (617) 262-5200. Fax: (617) 262-4021. E-mail: pstashenko@forsyth.org.

plasma liquid media. The cells were centrifuged at $7,000 \times g$ for 15 min and were resuspended in prerduced anaerobically sterilized Ringer's solution under the influx of nitrogen. The final concentration of each organism was determined by spectrophotometry, and the four pathogens were mixed to yield a concentration of 10^{10} cells of each pathogen/ml in 0.01 g of methylcellulose/ml. Following infection, the teeth were sealed with composite resin (Zenith, Englewood, N.J.) to prevent superinfection of pulp tissue with microorganisms from the oral cavity.

Abscess scoring and body weight measurements. Grossly evident orofacial abscesses were scored as positive or negative following visual examination. Body weight was measured on day 0 and again on the day of sacrifice. Spleen weights were determined at sacrifice.

Quantification of bone destruction. Mandibles were dissected free of soft tissue. The left hemimandibles were fixed in 5% neutral formalin and were decalcified in EDTA (10% [wt/vol] in 0.1 M Tris, pH 6.96) for histology. Six-micrometer paraffin sections were cut and stained with hematoxylin and eosin. The sections were encoded and evaluated by an observer blinded to the genotype. Sections which included the crown and distal root of the mandibular first molar, and which exhibited a patent root canal apex representing the central portion of the pulp and root canal, were selected for analysis. A minimum of three sections per tooth were evaluated histomorphometrically with an Optimas Bioscan image analysis system. The largest values of periapical lesion size, in square millimeters, from lower first molars were used as the measures of tissue destruction for each animal.

Inflammatory tissue preparation. Periapical tissues surrounding the mesial and distal root apices of the mandibular first molar were carefully extracted with surrounding bone in a block specimen under a surgical microscope. The gingiva, oral mucosa, and tooth crown were dissected free of the samples and discarded. Periapical tissues were rinsed in phosphate-buffered saline, freed of clots, weighed, and immediately frozen in dry ice-ethanol. Tissues were stored at -70°C until protein extraction. For extract preparation, frozen samples were ground in a precooled sterile mortar and pestle, and the tissue fragments were dissolved in 800 μl of lysis buffer consisting of 100 μl of bovine serum albumin per ml (fraction V; Sigma), 100 μl of Zwittergent-12 per ml (Boehringer Mannheim), 50 μl of gentamycin per ml (Life Technologies), 10 mM HEPES buffer (Life Technologies), 1 μg of leupeptin per ml (Sigma), and 0.1 μM EDTA (Fisher Scientific, Pittsburgh, Pa.) in RPMI 1640 (Mediatech, Herndon, Va.), as previously described (29). The incubation mixture was placed on ice and sonicated for 20 to 30 s. The supernatant was collected and stored at -70°C .

Stimulation of peritoneal macrophages in vitro. Resident peritoneal macrophages were prepared as previously described by Nacy and Osterman, with modifications (20). Briefly, macrophages were obtained from mice after intraperitoneal injection of 5 ml of culture medium consisting of RPMI 1640 supplemented with 10% fetal bovine serum (Mediatech), 5 U of heparin per ml (Sigma), 50 U of penicillin per ml, and 50 μg of streptomycin per ml (Sigma). The peritoneal lavage was pooled from three to six mice, counted, and centrifuged at $500 \times g$ for 10 min at room temperature. Peritoneal macrophages were resuspended at 10^6 cells/ml in culture medium without heparin, and 1-ml cultures were conducted at 37°C in 5% CO_2 in moist air in 24-well plates (Costar, Cambridge, Mass.).

After 2 h of incubation, macrophages were washed three times with culture medium to remove nonadherent cells and were stimulated with 10^7 formalinized bacteria of each of the pathogenic strains above per ml, as well as with a mixture of the four bacteria or *Escherichia coli* LPS (10 $\mu\text{g}/\text{ml}$; Sigma) as a positive control. The cell culture medium was harvested after 24 h, aliquoted, and stored at -70°C .

ELISA. Assays for cytokines used commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. The ELISA kits included IL-1 α (Endogen, Cambridge, Mass.), IL-1 β , TNF- α , IL-10, IL-12, and gamma interferon (IFN- γ) (all from BioSource International, Camarillo, Calif.). The concentration of each cytokine present in samples was calculated with reference to a standard curve that was constructed by using recombinant cytokines provided with each kit. Results were expressed as picograms of cytokine/milligram of periapical tissue or as picograms of cytokine/ml.

Statistical analysis. Areas of bone destruction and ELISA data were analyzed by the nonpaired Student's *t* test.

RESULTS

Bone destruction in TLR4-deficient mice. The effect of a functional deficiency in TLR4 on infection-stimulated bone resorption was assessed in C3H/HeJ and wild-type C3H/HeOuJ mice. Animals ($n = 10/\text{group}$) were subjected to surgical pulp exposure and were infected with a mixture of four anaerobic pathogens that are prevalent in endodontic infections. The animals from both groups were sacrificed 21 days after infection. The amount of periapical tissue destruction was determined by histomorphometry. Figure 1 shows a representative histological section of periapical bone destruction in TLR4-deficient C3H/HeJ and wild-type control C3H/HeOuJ

FIG. 1. (A) Photomicrograph of periapical inflammatory lesion associated with a tooth in a TLR4-deficient LPS-hyporesponsive C3H/HeJ mouse 21 days after pulpal exposure and anaerobic infection of the root canal system. (B) Photomicrograph of periapical inflammatory lesion associated with a tooth in a wild-type control C3H/HeOuJ mouse 21 days after pulpal exposure. Note the extent of bone resorption. Hematoxylin and eosin stain; magnification, $\times 200$. DR, distal root of mandibular first molar; B, bone; PL, periapical lesion. Arrows indicate limits of bone resorption.

mice. As shown in Fig. 2, significant bone resorption occurred in both infected C3H/HeJ and C3H/HeOuJ mice. Note that the bone resorption indicated for uninfected mice in fact represents the area of the normal periodontal ligament space. However, the mean area of bone resorption in TLR4-deficient C3H/HeJ was significantly reduced, by 43% compared to C3H/HeOuJ control mice ($P < 0.001$). This result demonstrates that an absence of TLR4 function results in decreased infraosseous bone destruction in response to infection.

Expression of cytokines in periapical tissues. Bone resorptive cytokine expression in inflammatory periapical tissues was assessed in TLR4-deficient C3H/HeJ and control C3H/HeOuJ mice by ELISA. As shown in Fig. 3, uninfected C3H/HeJ and C3H/HeOuJ mice showed consistently low levels of IL-1 α , IL-1 β , and TNF- α expression. However, C3H/HeOuJ showed significantly increased production of IL-1 α , approximately threefold greater than in C3H/HeJ mice ($P < 0.01$). IL-1 β was also higher in wild-type C3H/HeOuJ mice, although the difference was less profound. Of note, there was no significant difference in TNF- α , which is often coordinately regulated with IL-1 (11, 13).

Th1-type regulatory cytokines, such as IFN- γ and IL-12, are

FIG. 2. Infection-stimulated bone resorption in TLR4-deficient C3H/HeJ and wild-type C3H/HeOuJ mice. Error bars indicate standard errors of the means (SEM). Note that the indicated resorption in uninfected controls represents the area of the normal periodontal ligament space. Difference between infected C3H/HeJ and C3H/HeOuJ mice was analyzed by nonpaired Student's *t* test. ***, $P < 0.001$.

proinflammatory and have been reported to increase LPS-induced IL-1 expression, whereas Th2-type cytokines, such as IL-10, are antiinflammatory and decrease IL-1 (28). As shown in Fig. 4, uninfected C3H/HeJ and C3H/HeOuJ mice also showed low levels of baseline expression of IFN- γ and IL-12. LPS-responsive control mice produced significantly increased amounts of IL-12 compared to TLR4-deficient LPS-hyporesponsive mice ($P < 0.05$). In contrast, there was no significant difference in IFN- γ production in these two strains. The baseline expression of the Th2-type cytokine IL-10 in uninfected mice was very high compared to the Th1 cytokines. However, there was no significant difference in the levels of IL-10 in the two strains in response to infection.

Cytokine expression by TLR4-deficient macrophages in vitro. Macrophages express several TLRs, including TLR4, and are large-scale producers of inflammatory and bone resorptive cytokines (3). Cytokine expression was therefore examined in peritoneal macrophages from two different TLR4-deficient strains, C3H/HeJ and C57BL/10ScNcr, and was compared to the corresponding wild-type strains. As shown in Fig. 5A, wild-type C3H/HeOuJ macrophages produced significantly higher levels of IL-1 α than TLR4-deficient C3H/HeJ

FIG. 3. Quantitation of bone resorptive cytokines in periapical lesions by ELISA. Results are expressed as picograms of cytokine/milligram of periapical tissue. Error bars indicate SEM. Differences between infected C3H/HeJ and C3H/HeOuJ mice were analyzed by nonpaired Student's *t* test. *, $P < 0.05$; ***, $P < 0.001$.

FIG. 4. Quantitation of regulatory cytokines in periapical inflammatory lesions by ELISA. Error bars indicate SEM. Differences between infected C3H/HeJ and C3H/HeOuJ mice were analyzed by nonpaired Student's *t* test. *, $P < 0.05$.

macrophages in response to all stimulants. Similarly, wild-type C57BL/10ScN macrophages also exhibited higher IL-1 α production than TLR4-deficient C57BL/10ScNcr macrophages in response to all stimulants (Fig. 5B). In addition, an essentially identical pattern of IL-1 β expression was also obtained in these two TLR4-deficient mouse strains (Fig. 6A and B). These data suggest that the two strains C3H/HeJ and C57BL/10ScNcr are equally hyporesponsive to both whole bacteria and bacterial LPS.

Other cytokine responses of TLR4-deficient macrophages are summarized in Fig. 7. As indicated, wild-type C3H/HeOuJ macrophages again produced higher levels of TNF- α , IL-10, and IL-12 compared to TLR4-deficient C3H/HeJ mice in response to all stimulants. Somewhat surprisingly, this response pattern was observed to both gram-negative (*P. intermedia* and *F. nucleatum*) and gram-positive (*S. intermedius* and *P. micros*) pathogens. These data confirm the in vivo findings with periapical inflammatory tissues and also suggest that TLR4-deficient mice have decreased expression of proinflammatory and regulatory cytokines to both gram-negative and gram-positive bacteria.

Effect of TLR4 deficiency on disseminating infections. In previous studies, we demonstrated that RAG2 SCID and B-cell-deficient mice exhibit dentoalveolar abscess development, sepsis, splenomegaly, and cachexia in the absence of anti-bacterial antibody production following anaerobic infection in this model (36; L. Hou, H. Sasaki, and P. Stashenko, submitted for publication). The effects of TLR4 deficiency on infection dissemination and antibody production were therefore assessed. Neither TLR4-deficient C3H/HeJ nor control C3H/HeOuJ mice exhibited evident dentoalveolar abscesses at any time up to sacrifice on day 21 (data not shown). In addition, there was no loss of body weight (C3H/HeJ, 25.0 g [day 0] and 26.2 g [day 21]; C3H/HeOuJ, 27.1 g [day 0] and 27.8 g [day 21]) or splenomegaly (C3H/HeJ, 0.10 g; C3H/HeOuJ, 0.09 g) observed in either strain, suggesting that TLR4 function is unrelated to infection dissemination.

As noted, antibody is protective against disseminating infections in this model (L. Hou, H. Sasaki, and P. Stashenko, submitted for publication). The levels of antibody produced against the infecting pathogens were therefore assessed in C3H/HeJ and control C3H/HeOuJ mice. As shown in Fig. 8, there was no difference between the two strains in the levels of antibody against any of the four pathogens. Thus, TLR4 deficiency does not appear to have a significant impact on antibody production against these bacteria.

FIG. 5. IL-1 α expression in response to bacterial stimulants in vitro by peritoneal macrophages from two TLR4-deficient strains, C3H/HeJ (A) and C57BL/10ScNcr (B), compared to the corresponding wild type. Error bars indicate SEM. Differences in IL-1 α between TLR4-deficient macrophages and wild-type macrophages were analyzed by nonpaired Student's *t* test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$. *P. int.*, *P. intermedia*; *F. nuc.*, *F. nucleatum*; *S. int.*, *S. intermedium*; *P. mic.*, *P. micros*; Bac-mix, bacteria mixture.

DISCUSSION

TLR4 transduces responses to LPS leading to activation of NF- κ B and AP-1 and to the induction of proinflammatory cytokines (10, 14, 19, 43, 45). However, the effect of a deficiency in TLR4 function on bone destruction and dentoalveolar abscess development induced by a mixed anaerobic infection is not known. In the present study, we demonstrate that LPS-hyporesponsive C3H/HeJ mice had significantly reduced infection-stimulated alveolar bone destruction compared to wild-type C3H/HeOuJ mice. The decreased bone destruction was directly correlated with reduced expression of the bone resorptive cytokines IL-1 α and IL-1 β , as well as the Th1-inducing cytokine IL-12. As shown previously in this model, IL-1 levels correlate with bone destruction (12, 38), and IL-1 receptor antagonist inhibits bone resorption in vivo (30). IL-1 is also implicated in alveolar bone resorption in periodontal disease (2, 15, 18, 29).

The inbred mouse strain, C3H/HeJ, was initially characterized as LPS hyporesponsive more than 30 years ago (9, 31). A cardinal feature of this strain is its resistance to septic shock induced by exposure to high doses of LPS (32). On the cellular level, macrophages and fibroblasts fail to develop an activation phenotype or die when exposed to high concentrations of LPS (21). B lymphocytes do not respond, or are hyporesponsive, to the mitogenic, adjuvant, and immunogenic properties of LPS. Recently, a point mutation in the signaling domain of *Toll*-like receptor 4 was proposed to underlie hyporesponsiveness (22).

FIG. 6. IL-1 β expression in response to bacterial stimulants in vitro by peritoneal macrophages from two TLR4-deficient strains, C3H/HeJ (A) and C57BL/10ScNcr (B), compared to the corresponding wild type. Error bars indicate SEM. Differences between TLR4-deficient macrophages and wild-type macrophages were analyzed by nonpaired Student's *t* test. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$.

FIG. 7. Inflammatory and regulatory cytokine expression in response to bacterial stimulants *in vitro* by peritoneal macrophages from TLR4-deficient C3H/HeJ versus wild-type C3H/HeOuJ mice. Hatched bars, TLR4-deficient C3H/HeJ mice; black bars, wild-type C3H/HeOuJ mice. Error bars indicate SEM. Differences in cytokine production were analyzed by nonpaired Student's *t* test. *, $P < 0.05$; **, $P < 0.01$. *P. int.*, *P. intermedia*; *F. nuc.*, *F. nucleatum*; *S. int.*, *S. intermedius*; *P. mic.*, *P. micros*; Bac-mix, bacteria mixture.

Transfection of 393 cells with mutant C3H/HeJ TLR4 failed to confer LPS responsiveness, in contrast to wild-type TLR4 (10), demonstrating that this point mutation prevented TLR4-mediated signaling. TLR4 knockouts have a phenotype that is closely similar to that of C3H/HeJ mice (10). In this regard, the macrophages of both strains produce minimal amounts of TNF- α , and B cells fail to proliferate in response to LPS.

In the present study, as anticipated, the expression of inflammatory cytokines was significantly depressed *in vivo* and *in vitro* in TLR4-deficient strains. Somewhat surprisingly, these responses were similarly reduced in TLR4-deficient macrophages to both gram-positive and gram-negative bacteria *in vitro*. This finding suggests that TLR4, although clearly in-

involved in transducing responses to LPS, may not be specific for this ligand, but may possess broader specificity for other structures on gram-positive bacteria. Consistent with this finding, a recent report showed a severely impaired responsiveness in TLR4-deficient macrophages to gram-positive bacterial lipoteichoic acid (LTA), suggesting that TLR4 recognizes both LTA and LPS (34). Moreover, the recent demonstration that TLR2 transduces signals to gram-positive bacterial cell wall components peptidoglycan and LTA (27, 44), in addition to its earlier-reported stimulation by LPS (22), is consistent with the interpretation that TLRs in general may be promiscuous in terms of their ligand binding and activation properties.

While the data that C3H/HeJ mice are functionally TLR4

FIG. 8. Antibody production in TLR4-deficient C3H/HeJ and wild-type C3H/HeOuJ mice. Levels of antibody produced against *P. intermedia*, *F. nucleatum*, *S. intermedius*, and *P. micros* were determined by ELISA. Error bars indicate SEM.

deficient are compelling, there is evidence that mutations in other genes may also be involved in LPS hyporesponsiveness in this strain (7, 33). Recently, a mutation was identified in the Ran/TC4 GTPase (*Lps^d/Ran*) gene of C3H/HeJ that correlates with LPS hyporesponsiveness (41). Ran/TC4 is closely linked to the TLR4 locus and may be involved in downstream signaling events in LPS responses. Further studies are required to precisely define the role of these additional loci in LPS responsiveness in the C3H/HeJ mouse.

The consequence of diminished inflammatory cytokine expression on infection resistance is complex. In severe gram-negative infections or following a lethal endotoxin dose, LPS-hyporesponsive C3H/HeJ mice typically show enhanced survival, since they produce lower levels of IL-1, TNF- α , and IFN- γ and do not experience septic shock (37). C3H/HeJ also show increased resistance to infection with *Pseudomonas aeruginosa* (37) and *Mycobacterium paratuberculosis* (35) compared to wild type. In contrast, C3H/HeJ is highly susceptible to infection with certain gram-negative pathogens, including *Rickettsia tsutsugamushi* (8), *Rickettsia akari* (1), *Salmonella typhimurium* (21, 39), *Ehrlichia risticii* (40), and *E. coli* (4, 6). Of interest, clearance of *E. coli* was enhanced by activation of liver Kupffer cells and peritoneal macrophages in vivo with *Mycobacterium bovis* BCG and in vitro with IFN- γ , but not with LPS. Pretreatment of C3H/HeJ mice with a combination of IL-1 and TNF- α also restored the killing of *E. coli*. This suggests that an LPS-initiated, cytokine-mediated response is involved in host defense mechanism against sublethal challenge with *E. coli*, and possibly other gram-negative pathogens. Taken together, cytokine-mediated responses in bacterial infections of C3H/HeJ may have protective or deleterious effects, depending on the pathogen and the bacterial load (6).

Neither C3H/HeJ nor wild-type control C3H/HeOuJ mice exhibited evident dentoalveolar abscesses in these studies. There was also no loss of body weight or splenomegaly in these two strains, indicating that no disseminating infections had occurred. As demonstrated in our earlier work, an antibody-mediated mechanism plays an important role in preventing disseminating infections in this model (L. Hou, H. Sasaki, and P. Stashenko, submitted for publication). In the present study, antibody responses to the pathogenic challenge were similar in

the two strains, which may account for the lack of infection dissemination observed.

The Th1-type regulatory cytokine IFN- γ activates macrophages and has been reported to increase LPS-induced IL-1 and TNF- α expression, whereas Th2-type mediators (IL-4, IL-10, and IL-13) are inhibitory (28). Th2-type cytokines in particular appear to inhibit IL-1 expression and bone destruction in the periapical model (25). In the present study, a modest elevation in IL-12 was observed in wild-type versus C3H/HeJ mice, although there was no consequent increase in IFN- γ . No significant differences were seen in the level of IL-10 in the two strains. Taken together, these findings suggest that the higher expression of IL-1 α and IL-1 β in wild-type mice is likely a direct effect mediated via TLR4 rather than indirect, exerted through modulation by Th1- or Th2-type cytokines.

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