Supporting Information

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SI Materials and Methods

Mice. Six- to 8-wk-old female C57BL/6 were purchased from Charles River Laboratory. C3H/HeJ (TLR4-deficient strain), C3H/HeOuJ (TLR4 normal strain), and *Il1r1*$^{-/-}$ mice were obtained from The Jackson Laboratories. *Tlr4*$^{-/-}$, *Tlr7*$^{-/-}$, *Tlr9*$^{-/-}$, and *Myd88*$^{-/-}$ mice were gifts from Shizuo Akira (1) and were maintained on a C57BL/6 background at the University of California San Diego. All animal protocols received prior approval by the institutional review board.

Reagents. Zoledronate (ZLD, AK Scientific), alendronate (ALD), and clodronate (Sigma) were endotoxin-free as determined by the Limulus Amebocyte Lysate test (Charles River Laboratory). In vitro stimulation studies were performed with the indicated doses of LPS (Sigma), heparan sulfate (Sigma), IL-1α (eBioscience), TLR7 ligand [1V136; synthesized as previously described (2)], Pam3CSK4 (Invivogen) and poly(I:C) (Invivogen), and single-stranded phosphorothioate immunostimulatory oligonucleotide (Tri-Link Biotechnology) (3). Human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats obtained from the San Diego Blood Bank.

In Vivo Models. In the peritonitis model, mice were injected intraperitoneally with the 0.8 mg/mouse ALD in 100 μL vehicle (PBS). Peritoneal cells were recovered by lavage with 3 mL cold PBS. Cells were counted with a Guava Personal Cytometer (Millipore) and differentiated by Wright-Giemsa staining or by flow cytometry. Bone marrow chimeras were generated as described previously (4). For adjuvant studies, WT mice were injected subcutaneously in the flank with 10 μg ZLD and/or 30 μg ovalbumin in 200 μL vehicle. Antigen-specific IgG levels as well as antigen specific IFN-γ secretion by splenocytes were determined as described previously (5).

In Vitro Cytokine Assays. Bone marrow-derived macrophages (BMDM) and human PBMCs were prepared as described previously (5). BMDM (1.5×10^5 per well in 96-well plates) or human PBMCs (2.5×10^5 per well in 96-well plates) were pretreated with nitrogen bisphosphonates (first treatment), followed by additional LPS or other Toll-like receptor ligands for another 24 h. In the indicated experiment, farnesyl-pyrophosphate (Sigma) was added during the first treatments as indicated. Mesothelial cells were isolated as previously described (6). The mesothelial cells (1×10^4 per well in 96-well plates) were pretreated with vehicle or 10 μM ZLD followed by murine IL-1α for an additional 24 h. Harvested supernatants were assayed for murine IL-6 and KC, human IL-8 (BD Bioscience), and murine IFN-γ (Antigenix America) by ELISA.

Quantitative RT-PCR. RNA was isolated and levels of transcripts were determined as previously described (7). The following primer sets and probes were used in the experiment: 5′-TGCCTTCATTTATCCCTTGAA-3′ (forward), 5′-TTACTACATTCAGCCAAAAAG-3′ (reverse), and 5′-TCTGGTCC-3′ (probe) for IL-6; 5′-GGACATTCGAAACCAAGCAT-3′(forward), 5′-CCAGTGCCTTCTTTAGTTGCATT-3′(reverse) and 5′- TTCTCCTG-3′ (probe) for IRAK-M; 5′-ACCAGAGGCATACAGGGACA-3′(forward), 5′-CTAAGGCCAACCGTGAAAAG-3′ (reverse) and 5′-CAGCCTGG-3′(probe) for β-actin.

Immunoblot. Lysates from treated BMDM were immunoblotted for IL-1 receptor-associated kinase-M (IRAK-M), IRAK-1 (Abcam), JNK and phospho-JNK (Cell Signaling), and β-actin (Sigma) (4).

Statistics. The statistical differences for multiple comparisons were analyzed by one-way ANOVA with Dunnett's or Bonferroni post hoc test, as indicated. Analyses for pair-wise comparisons were made using Mann-Whitney *U* tests. All analyses were performed using Prism software (version 5.0, GraphPad Software, Inc.). A value of $P < 0.05$ was considered statistically significant.

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