MyD88 Is Pivotal for the Early Inflammatory Response and Subsequent Bacterial Clearance and Survival in a Mouse Model of Chlamydia pneumoniae Pneumonia*

Received for publication, March 23, 2005, and in revised form, June 8, 2005 Published, JBC Papers in Press, June 17, 2005, DOI 10.1074/jbc.M503225200

Chlamydia pneumoniae is the causative agent of respiratory tract infections and a number of chronic diseases. Here we investigated the involvement of the common TLR adaptor molecule MyD88 in host responses to C. pneumoniae-induced pneumonia in mice. MyD88-deficient mice were severely impaired in their ability to mount an acute early inflammatory response toward C. pneumoniae. Although the bacterial burden in the lungs was comparable 5 days after infection, MyD88-deficient mice exhibited only minor signs of pneumonia and reduced expression of inflammatory mediators. MyD88-deficient mice were unable to up-regulate pro-inflammatory cytokines and chemokines, demonstrated delayed recruitment of CD8+ and CD4+ T cells to the lungs, and were unable to clear the pathogen from their lungs at day 14. At day 14 the MyD88-deficient mice developed a severe, chronic lung inflammation with elevated IL-1β and IFN-γ leading to increased mortality, whereas wild-type mice as well as TLR2- or TLR4-deficient mice recovered from acute pneumonia and did not show delayed bacterial clearance. Thus, MyD88 is essential to recognize C. pneumoniae infection and initiate a prompt and effective immune host response against this organism leading to clearance of bacteria from infected lungs.

A compelling body of evidence indicates that the host critically relies upon Toll-like receptors (TLRs)1 to sense the presence of foreign pathogens and initiate both rapid and sustained defenses (6, 7). Association of pathogen-associated molecular patterns (PAMPs) with their cognate TLRs initiates signaling that requires assemblies of heteromeric complexes composed of dimerized TLRs, together with one or more adaptor molecules for effective downstream transmission and transcriptional responses that defend against the pathogen and activate a variety of other defensive mechanisms.

Most (but not all) TLR signaling involves the adaptor molecule MyD88 (myeloid differentiation factor 88), but MyD88-independent responses are important though poorly understood. MyD88−/− mice are unable to mount a vigorous Th1 response, and instead Th2 polarization predominates. Whereas some studies indicate that long term adaptive responses may be preserved, other data support a model wherein adaptive responses are critically dependent upon intact TLR signaling, and hence MyD88 (8). Furthermore, in some cases it appears that late activation of inflammatory cytokines can occur in the absence of MyD88. Mice deficient in MyD88 are markedly susceptible to systemic challenge with Staphylococcus aureus or Listeria monocytogenes (9–12), but are partially protected from polymicrobial peritonitis, possibly because of attenuation of an injurious inflammatory response (13). MyD88-dependent responses were essential for effective lung defenses against pulmonary infection with Pseudomonas aeruginosa but not S. aureus (14). Therefore, the consequences of MyD88 deficiency appear to be both pathogen and tissue-specific, suggesting substantial complexity in innate defenses, whereby essential or redundant roles for specific components are dependent both on the nature and the site of infection (14).

Several studies have investigated the potential interaction of C. pneumoniae with TLRs in vitro. Chlamydial heat shock protein 60 (HSP60) has been shown to signal via TLR4 (15, 16) and yet undefined non-LPS components of C. pneumoniae may stimulate cytokine release involving TLR2 (17). The utilization

---

*This work was supported by Grants HL 66436 and AI 058128 (to M. A.) from the National Institutes of Health and by Public Health Service Grant AI-3248 (to E. M. P.) from NIADD, National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**To whom correspondence should be addressed: Cedars-Sinai Medical Center, Division of Infectious Diseases and Immunology, 8700 Beverly Blvd., Rm. 4220, Los Angeles, CA 90048. Tel.: 310-423-1034; Fax: 310-423-8284; E-mail: moshe.arditi@cshs.org.

---

† Members of the family Chlamydiaceae are obligate intracellular pathogens that cause a wide variety of human diseases that include ocular, pulmonary, and genital infections (1, 2). Within this family Chlamydia pneumoniae is in particular clinically important, not only because it has a worldwide distribution and is responsible for about 10% of community-acquired pneumonia and 5% of sinusitis and bronchitis, but also because it has been strongly implicated as a major contributor to vascular diseases such as atherosclerosis (2–4) and chronic lung disease such as asthma (5).

The abbreviations used are: TLRs, Toll-like receptors; MyD88, myeloid differentiation factor 88; WT, wild-type; BAL, bronchoalveolar lavage; TIR, Toll/IL-1 receptor; IFU, inclusion forming unit; TRIF, Toll/IL-1 receptor domain-containing adaptor inducing interferon-β; TRAM TRIF-related adaptor molecule; IL, interleukin; MOI, multiplicity of infection; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; MEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; IFN, interferon; PMN, polymorphonuclear cell; FACs, fluorescent-activated cell sorter; LPS, lipopolysaccharide.

---

29242 This paper is available online at http://www.jbc.org
of TLRs by live C. pneumoniae is not completely understood. The induction of interferon-γ (IFN-γ), a crucial step in the elimination of C. pneumoniae and both MyD88-dependent and MyD88-independent signaling were found to be important and complementary for IFN-γ expression in macrophages after intracellular bacterial infection (18). To clarify the role of MyD88 signaling in host defense against C. pneumoniae, we inoculated MyD88−/− and wild-type mice intranasally with C. pneumoniae and compared the immune response and survival among the genotypes. We found that MyD88 is critically important for mounting a rapid and effective lung defenses against C. pneumoniae. In the absence of MyD88, an acute host defense against infection by C. pneumoniae does not occur, and infected mice rapidly succumb to infection. Surprisingly, we also found that a robust MyD88-independent inflammatory response does develop considerably later. MyD88-deficient mice were unable to clear bacteria and showed a severe, chronic lung inflammation with elevated systemic IL-1β and local IL-1β and IFN-γ concentrations, and intact adaptive immune responses with similar levels of C. pneumoniae-specific Abs to wild-type mice. These responses nevertheless were associated with decreased survival in C. pneumoniae-infected MyD88-deficient mice.

**EXPERIMENTAL PROCEDURES**

**Mice**—MyD88−/−, TLR2−/−, and TLR4−/− mice were kindly provided by Dr. S. Akira (Osaka University, Osaka, Japan). A homogenous population was established by backcrossing MyD88−/− mice to C57BL/6 mice for more than five generations. Mice population was established by backcrossing MyD88−/−, TLR2−/−, and TLR4−/− mice as indicated. For the preparation of alveolar macrophages, mice were sacrificed, and the trachea was surgically exposed. A 22G intravenous catheter (BD Biosciences, San Jose, CA) was inserted and secured with a suture, followed by injection of 5 × 10^5 ml of ice-cold HBSS (Invitrogen) containing 2 mM EDTA and collection of the aspirate. Cells were harvested by centrifugation, washed once in DMEM/10% FCS/gentamicin and counted. We routinely isolated 1.5–3.0 × 10^6 cells per animal. Macrophages were transferred to 96-well tissue culture plates at a density of 2.5 × 10^4 cells/well. After 4 h, macrophages were washed once to remove non-adherent cells and stimulated for 24 h in DMEM/10% FCS/gentamicin as indicated.

**Cytokine ELISAs and Measurements of C. pneumoniae-specific Total IgG**—The cytokine concentrations in the serum, BAL, lung homogenates, or culture supernatant were analyzed using OptEIA™ Mouse IL-1β, IL-12p40, and IL-6 (BD Biosciences, Franklin Lakes, NJ), DuoSet™ MCP-1 (JCE/CCL2), MIP-2 (R&D Systems), and Mouse IFN-γ ELISA (eBioscience). An ELISA was used to determine serum titers to C. pneumoniae-specific total IgG as described previously (22).

**Flow Cytometry Analysis**—The lymphocytes distributed in the lungs after infection were analyzed by flow cytometry of lung homogenates. Briefly, lymphocytes were isolated by digesting the lung tissue at 37 °C for 1 h with RPMI medium containing 1 mg/ml collagenase A (Roche Applied Science, Indianapolis IN) and 50 units/ml DNase and filtering through a 100-μm cell strainer (BD Falcon, Bedford, MA). Erythrocytes were depleted by lysis buffer before further analysis. Isolated single cells were stained with appropriate antibodies against cell surface markers. Specifically, for T cell phenotyping, lymphocytes were stained with fluorescein isothiocyanate-conjugated anti-CD8α mAb, PE-conjugated anti-CD4 mAb, or APC-conjugated anti-CD3e mAb (BD Biosciences). Data of multicolor-stained lymphocytes were then collected on a FACScalibur™ flow cytometer (BD Biosciences) and analyzed using CELLQuest™ software.

**Statistics**—Data are reported as mean values ± S.D. Statistical significance was determined by Student’s t test or Mann-Whitney U test. Statistical significance for survival studies was determined by Fisher’s exact test. A p value of less than 0.05 was considered significant.

**RESULTS**

**MyD88 Deficiency Protects Mice from Acute Lung Inflammation on Day 5 of a C. pneumoniae Infection but Leads to Severe Chronic Lung Inflammation at Days 14 and 35**—To investigate the role of MyD88 in host defense against an acute C. pneumoniae infection MyD88−/− and MyD88+/+ mice were inoculated intranasally with C. pneumoniae and sacrificed at day 5 of postinoculation. Macroscopically, gross examination of the infected lungs demonstrate severe inflammation as apparent by more consolidation with pale, tan firm cut surfaces in MyD88−/− mice, whereas MyD88+/+ mice did not show macroscopic signs of inflammation (Fig. 1A). On day 5, the lungs revealed patchy interstitial and diffuse pneumonia with massive infiltration of neutrophils into lung tissue in MyD88−/− mice, whereas MyD88+/+ mice showed significantly less or no signs of lung inflammation (Fig. 1B). The lung inflammation severity score for MyD88+/+ was significantly higher (p < 0.01) in infected MyD88−/− mice compared with MyD88+/+ mice on day 5 (Fig. 1C). However, there was a dramatic reversal of the lung histopathology at days 14 and 35. Whereas MyD88+/+ mice had cleared the lung inflammation, MyD88−/− mice now developed a severe, diffuse, and chronic lung inflammation, with a significantly higher lung inflammation score (p < 0.05) compared with MyD88+/+ mice (Fig. 1C). Histopathological analysis on day 35 revealed that all of the MyD88−/− mice cleared the lung inflammation, whereas 50% of MyD88+/+ mice that survived still had severe, chronic lung inflammation (Fig. 1B). Whereas the acute inflammation seen at day 5 in the lungs of MyD88−/− mice showed massive polymorphonuclear cell (PMN) infiltration, the inflammation observed on day 14 in the lungs of MyD88−/− mice showed mostly lymphocytic cells and some plasma cell infiltration but lacked PMN infiltration (Fig. 1D).
MyD88 in inflammatory host responses against *C. pneumoniae* infection, we measured the body weight of MyD88^−/−^ and WT mice before inoculation and 5 and 14 days after inoculation with *C. pneumoniae*. Infection resulted in a significant decrease of relative body weight in MyD88^−/−^ mice at day 5 after inoculation (p < 0.05) (compared with the body weight of mice prior to infection), but no reduction in relative body weight was observed in MyD88^+/+^ mice at day 5 compared with the body weight of mice prior to infection (Fig. 2A).

Consistent with the development of lung inflammation, MyD88^−/−^ mice demonstrated an increase in lung weight compared with power magnification of *C. pneumoniae*-infected lungs of MyD88^+/+^ and WT mice at days 5 and 14 postinfection. Lungs from WT mice show intense infiltration with PMNs at day 5 after inoculation and clearance of inflammation by day 14. On the other hand, lungs from MyD88^−/−^ mice show no inflammation at day 5, but severe inflammation at day 14 characterized by intense lymphocytic infiltration with some plasma cells and lack of PMNs.
MyD88<sup>−/−</sup> mice at day 5 (p < 0.05). However, at day 14, lung weight from MyD88<sup>−/−</sup> mice returned to base line coinciding with a recovery from acute inflammation and edema, whereas MyD88<sup>−/−</sup> mice demonstrated an increased lung weight at day 14 consistent with development of severe lung inflammation (Fig. 2B). These findings are supported by a significantly increased number of inflammatory cells in the BAL of MyD88<sup>−/−</sup> mice compared with MyD88<sup>+/+</sup> mice at day 5 (p < 0.05) (Fig. 2C). Whereas inflammatory cells of MyD88<sup>−/−</sup> mice were decreased by day 14 compared with day 5, we could not observe any differences in inflammatory cells in the BAL between the two genotypes at day 14 (Fig. 2C).

Next, we measured the concentrations of IFN-γ, IL-1β, and MIP-2 in serum, lung homogenates, and BAL from infected MyD88<sup>−/−</sup> or MyD88<sup>+/+</sup> mice at days 5, 14, and 35 (Fig. 3). At day 5, IFN-γ, IL-1β, and MIP-2 concentrations in lung homogenates or BAL were significantly higher in infected MyD88<sup>−/−</sup> mice compared with MyD88<sup>+/+</sup> mice, which were unable to mount an early inflammatory response (Fig. 3, A–C). Consistent with the observations with lung histopathology and bacterial burden, there was a dramatic reversal at day 14, when MyD88<sup>−/−</sup> mice cleared the inflammation and pro-inflammatory cytokines returned to baseline, whereas MyD88<sup>−/−</sup> mice developed a significant increase in IFN-γ and IL-1β levels locally (lung homogenate and BAL) (Fig. 3, A and B). However, MIP-2 levels in lung homogenate and BAL samples of MyD88<sup>−/−</sup> mice remained low even at day 14, consistent with the lack of PMN infiltration in the lung inflammation observed at this day (Figs. 3C and 1D). At day 35, inflammatory cytokine levels in serum or lung homogenate were normal in control MyD88<sup>+/+</sup> mice, whereas they remained elevated in MyD88<sup>−/−</sup> mice (Fig. 3B). These findings suggest that MyD88 deficiency impairs host innate immunity, delays bacterial clearance from the lungs, leading to a late and chronic lung inflammation, which lacks infiltration of PMNs.

C. pneumoniae-infected MyD88<sup>−/−</sup> Mice Fail to Clear the Bacteria from Their Lungs at 14 Days, Which Leads to Increased Mortality—To investigate whether the delayed but severe and chronic inflammation in the lungs of MyD88<sup>−/−</sup> mice on days 14 and 35 was caused by delayed bacterial clearance and sustained high bacterial burden, we quantified C. pneumoniae IFU in the lungs of MyD88<sup>−/−</sup> and MyD88<sup>+/+</sup> mice at day 5 and day 14. There were no differences in the bacterial count in infected lungs between MyD88<sup>−/−</sup> and MyD88<sup>+/+</sup> mice on day 5 following inoculation (Fig. 4A). However, at day 14 there was a dramatic reduction in bacterial burden in the lungs of MyD88<sup>−/−</sup> mice, whereas MyD88<sup>−/−</sup> mice failed to clear bacteria from their lungs (Fig. 4A). The bacterial burden in the lungs of MyD88<sup>−/−</sup> mice on day 14 was comparable to the bacterial count on day 5 after inoculation (Fig. 4A). In addition, we observed ~45% mortality in C. pneumoniae-infected MyD88<sup>−/−</sup> mice (5 of 11 mice died) by day 35 whereas 100% of the infected MyD88<sup>+/+</sup> mice survived the infection (p < 0.01) (Fig. 4B). To determine whether antibody production was altered in MyD88<sup>−/−</sup> mice when challenged with C. pneumoniae, total IgG levels were determined in sera collected at 14 and 35 days. MyD88<sup>−/−</sup> mice produced the same titers of total C. pneumoniae-specific IgG as compared with MyD88<sup>+/+</sup> mice both at day 14 and day 35 (data not shown).

MyD88<sup>−/−</sup> Mice Demonstrate Delayed Recruitment of CD8<sup>+</sup> and CD4<sup>+</sup> T Cells to Lung Tissues Infected with C. pneumoniae—To further understand the mechanism of the reversal of the inflammation in the lungs of MyD88<sup>−/−</sup> mice on day 5 (early) versus day 14 (late), we determined the cellular constitution of the inflammatory cells in the lung homogenates in MyD88<sup>−/−</sup> and MyD88<sup>+/+</sup> mice following C. pneumoniae infection. The absolute number of lymphocytes reached maximal levels on day 5 in the lungs of MyD88<sup>−/−</sup> mice, but their recruitment was delayed in the lungs of MyD88<sup>−/−</sup> mice, and they reached maximal levels only on day 14. Consistent with

![Inflammatory response after C. pneumoniae infection is diminished in MyD88 deficient mice during acute infection but persists during chronic infection.](image-url)
the reversal of the lung inflammation at day 5 versus 14, the absolute number of CD4⁺ T cells and CD8⁺ T cells increased at day 5 in the lungs of MyD88⁺/⁻ mice (but not MyD88⁻/⁻ mice), and dramatic reversal occurred at day 14 when the number of lung CD4⁺ T cells and CD8⁺ T cells diminished in WT mice, while they now increased in MyD88⁻/⁻ mice (Fig. 5B).

MyD88⁻/⁻ and TLR2⁻/⁻ Macrophages Are Deficient in Cytokine Responses to Live C. pneumoniae Infection—To confirm the role of MyD88 in inflammatory host responses against C. pneumoniae infection in vitro, we isolated primary bone marrow-derived macrophage from MyD88⁺/⁻ and MyD88⁻/⁻ mice and infected them with live C. pneumoniae at an MOI of 3 and measured MCP-1, IL-6, and IL-12p40 release. C. pneumoniae infection induced significant amount of MCP-1, IL-6, and IL-12p40 release in WT but not in MyD88⁻/⁻ macrophages (Fig. 6A). However, macrophages isolated from wild-type and MyD88-deficient mice showed comparable ability in intracellular killing of C. pneumoniae in vitro (data not shown). To determine the upstream receptor involved in the recognition of C. pneumoniae, we isolated primary alveolar macrophages from TLR2⁻/⁻, TLR4⁻/⁻, and WT mice and infected them with live C. pneumoniae at an MOI of 3 for 24 h. While TLR4⁻/⁻ and WT alveolar macrophages responded to live C. pneumoniae stimulation by releasing IL-6, the TLR2⁻/⁻ macrophages were unresponsive, suggesting that at least in vitro TLR2 plays a role upstream of MyD88 in C. pneumoniae-induced cellular activation (Fig. 6B).

TLR2 or TLR4 Deficiency Is Not Associated with Delayed Bacterial Clearance or Delayed Lung Inflammation in C. pneumoniae Infection—We next investigated whether TLR2⁻/⁻ or TLR4⁻/⁻ mice have a defect in bacterial clearance and host defense against C. pneumoniae infection. TLR2⁻/⁻, TLR4⁻/⁻, or WT mice were inoculated intranasally with C. pneumoniae.
as described above and sacrificed at days 5 and 14 postinoculation. Lungs were examined histopathologically and quantitative cultures were performed. In contrast to MyD88−/−/H11002 mice, TLR2−/−/H11002, and TLR4−/−/H11002 mice were able to mount an acute inflammatory response early at day 5 and cleared the lung inflammation by day 14, similar to WT mice (Fig. 6C). There were no differences in the inflammation scores in lungs of WT mice versus TLR2−/− or TLR4−/− mice at days 5 or 14 (Fig. 6C). Furthermore, in contrast to MyD88−/− mice, TLR2−/−, or TLR4−/− mice were able to clear the bacteria from their lungs as well as the WT mice by day 14 (Fig. 6C). There was no increased mortality in the TLR2−/− or TLR4−/− mice compared with WT mice throughout the 35 days after infection (data not shown).

**DISCUSSION**

The major findings of these studies are that MyD88−/− mice fail to mount an effective lung defense against intrapulmonary challenge with *C. pneumoniae*. Our data implicate a central role for MyD88-dependent signaling in innate immune defense against *C. pneumoniae*. We found that both TLR2−/− and TLR4−/− mice were able to mount a successful host defense. However, MyD88-deficient mice were unable to up-regulate pro-inflammatory cytokines, demonstrated delayed recruitment of CD8+ and CD4+ T cells to the lung, did not recruit PMNs, were unable to clear the pathogen from their lungs, and exhibited greater mortality compared with wild-type, TLR2−/−, or TLR4−/− mice.

MyD88−/− and WT macrophages showed comparable ability to kill the *C. pneumoniae* in vitro. This finding is consistent with previous data showing that macrophage killing of *Listeria* was also found to be MyD88-independent (12). Importantly, a delayed MyD88-independent inflammatory response involving locally increased expression of IL-1β and IFN-γ did eventually develop at day 14 after inoculation, but in many cases was insufficient to prevent death. Levels of *C. pneumoniae*-specific total IgG antibodies in MyD88−/− mice were not different from those in MyD88−/− mice 14 and 35 days after intranasal inoculation, suggesting that MyD88−/− mice could mount an appropriate though ultimately ineffective adaptive immune response against *C. pneumoniae*. Collectively then, our findings implicate a critical role for MyD88-dependent signaling in mounting a rapid and effective innate immune response.
against pathogenic invasion by *C. pneumoniae* in this mouse model. These data are most consistent with the interpretation that the primary problem was an inability to produce proinflammatory cytokines and chemokines to recruit cell types to the site of infection that are known to be involved in eradication of *C. pneumoniae*, particularly cytotoxic CD8+ T cells. We also observed diminished PMN recruitment to the lungs of MyD88-deficient mice, an observation well described by many other studies (12, 14).

TLR2 and TLR4 heavily rely upon MyD88 to trigger cytokine responses, but we found that both TLR2- and TLR4-deficient mice were able to mount an immune response that successfully and efficiently countered the infectious challenge. These findings raise the question of how the host detects the presence of *C. pneumoniae*. One possibility is that TLR2 and TLR4 play redundant roles, and thus it is possible that TLR2/TLR4 double knock-out mice may have defects in host defenses against *C. pneumoniae* similar to those we observed with MyD88-null mice. Another possibility is that neither TLR2 nor TLR4 are involved in the recognition of this organism. Alternatively, in addition to TLRs, IL-1βR, and IL-18R also utilize MyD88 for signaling, raising the possibility that our results in MyD88−/− mice might have been caused by attenuated signaling triggered by these receptors rather than by TLR-mediated signals. This possibility is consistent with a recent report that also suggested that signaling by TLR2 or TLR4 may not be necessary for clearance of *C. pneumoniae* infection in mice after a low grade infectious challenge (23). Our current data now support these findings in high grade infectious challenge model as well.

Alternatively, MyD88-independent pathways such as the TRAM/TRIF pathway or pattern recognition receptors other than TLRs such as the intracytoplasmic nucleotide binding oligomerization domain (NOD) receptors may be important in some infections, or there may be significant redundancies in the response options available, such that these alternative signaling pathways may become activated in the absence of MyD88. Recently, an involvement of NOD1 signaling has been proposed for *C. pneumoniae*-induced NF-κB activation in vitro (24). It is also possible that TLR2, TLR4, and other pattern recognition receptors may form a redundant system for detecting *C. pneumoniae*, but that the downstream adaptor MyD88 that is common to these receptors is absolutely required for an appropriate innate defense. Available evidence is therefore most consistent with the interpretation that MyD88 is required for an effective host response to *C. pneumoniae*, but the identity of the upstream ligand(s) and the precise role of specific pattern recognition receptors must await the results of further investigation.

Despite these uncertainties, based on a large body of evidence, current models of innate immune defenses postulate a central role for TLR-mediated processes in pathogen detection, initiation of a rapid innate immune defense, and regulation of subsequent adaptive immune responses (6, 7) and thus make it difficult to rule out involvement of TLRs in host defense against *C. pneumoniae*. We note that most TLRs can utilize MyD88 to trigger responses, and we therefore suggest that our results are most likely because of abrogated pathogen detection mechanisms by one or more TLRs that utilize MyD88. In any event, our results clearly support the general model of pathogen detection and initiation of host defenses, and specifically demonstrate the central importance of MyD88 in mediating host defense against *C. pneumoniae*.

It appears that the inability of MyD88−/− mice to recruit appropriate immune effector cells to the lungs probably was a major contributor to the inability to clear pathogens from the lungs in MyD88−/− mice and reduced survival. Specifically, we found that MyD88−/− mice could neither produce inflammatory cytokines nor recruit CD8+ T cells to the lungs early in the course of the infection (i.e. within at least the first 5 days). However, later on (i.e. at 14 days), both inflammation and recruitment of CD8+ T cells were observed in the lungs of MyD88−/− mice. On the other hand, PMNs were not recruited either early or late in MyD88−/− mice, but were abundant in lungs of wild-type mice. Therefore, PMNs do not appear to be a significant contributor to the late chronic inflammation seen in MyD88−/− mice, but could be involved in rapid killing and clearance of pathogens from the lungs in the early phases of infection in WT mice. Rodriguez et al. (25) recently reported that PMNs actually facilitate replication of *C. pneumoniae* in epithelial cells, which would imply that lack of PMNs in the lungs of MyD88−/− mice would lead to a reduced bacterial load. However, we found that MyD88−/− mice had a defect in bacterial clearance from the lungs and had the same amount of bacteria at day 14 compared with day 5. Thus, lack of PMNs did not result in any decrease in bacterial load in the lungs of MyD88−/− mice at later days in our study. Whereas Rodriguez et al. (25) looked at early events at days 3 and 6 after infection our study addresses both early and late stages by investigating days 5, 14, and 35 after infection. We also found that MyD88-deficient mice have a delay in CD8+ T cell recruitment to the lung after infection. This finding directly implicates MyD88-dependent signaling in recruitment of CD8+ T cells to sites of infection. Because CD8+ T cells are known to be essential for resolution of *C. pneumoniae* infection (26), our observation of delayed CD8+ T cell recruitment to the lungs may provide an explanation for the reversal of phenotype that we observed in MyD88-deficient mice on days 5 and 14. Whereas previous studies have demonstrated no defects in MyD88-deficient mice in the total number of CD4+ and CD8+ T cells, and in their ability to produce IFN-γ after nonspecific activation (27), more recent studies have shown that MyD88-deficient mice have defects in their CD8+ T cell response to acute viral infections such as lymphocytic choriomeningitis virus (LCMV) (28, 29). Hence, our data are most consistent with the interpretation that the major reason MyD88−/− mice could not clear the pathogen from their lungs and developed a chronic inflammation was that an early localized proinflammatory response was lacking, PMNs were not recruited, and recruitment of CD8+ T cells into the infected lungs was delayed, that these factors conspired to allow the bacteria to flourish in the lungs of MyD88−/− mice, and that continued inability to clear the pathogen led to the delayed but intense lung inflammation and increased mortality.

We conclude that MyD88 critically regulates host defense to infectious challenge with *C. pneumoniae* in mice, that an effective, rapid innate immune response to clear the bacteria in this mouse model cannot be successfully mounted in the absence of MyD88, and that the result of MyD88 deficiency is chronic lung inflammation and higher mortality from an inability to effectively counteract *C. pneumoniae* infection.

Acknowledgments—We thank Terence M. Doherty for critical reading of the manuscript and editorial contribution. We also thank Polly Sun for excellent technical assistance.

REFERENCES
1. Belland, R., Ojcius, D. M., and Byrne, G. I. (2004) *Nat. Rev. Microbiol.* 2, 530–531.
2. Campbell, L. A., and Kuo, C. C. (2004) *Nat. Rev. Microbiol.* 2, 23–32.
3. Kuo, C. C., Jackson, L. A., Campbell, L. A., and Grayston, J. T. (1995) *Clin. Microbiol. Rev.* 8, 451–461.
4. Belland, R. J., Ouellette, S. P., Gieffers, J., and Byrne, G. I. (2004) *Cell Microbiol.* 6, 117–127.
5. Hansbro, P. M., Beagley, K. W., Horvat, J. C., and Gibson, P. G. (2004) *Pharmacol. Ther.* 101, 193–210.
6. Akira, S., and Takeda, K. (2004) *Nat. Rev. Immunol.* 4, 499–511.
7. Beutler, B. (2004) *Nature* 430, 257–263.
8. Doherty, T. M., and Arditi, M. (2004) J. Clin. Investig. 114, 1699–1703
9. Takeuchi, O., Hoshino, K., and Akira, S. (2000) J. Immunol. 165, 5392–5396
10. Seki, E., Tsutui, H., Tsuji, N. M., Hayashi, N., Adachi, K., Nakano, H., Futatsugi-Yumikura, S., Takeuchi, O., Hoshino, K., Akira, S., Fujimoto, J., and Nakashima, K. (2002) J. Immunol. 169, 3863–3868
11. Way, S. S., Kollmann, T. R., Hajjar, A. M., and Wilson, C. B. (2003) J. Immunol. 171, 533–537
12. Edelsten, B. T., and Unanue, E. R. (2002) J. Immunol. 169, 3869–3875
13. Prebeck, S., Kirschning, C., Durr, S., da Costa, C., Denath, B., Brand, K., Redeker, V., Wagner, H., and Metzke, T. (2001) J. Immunol. 167, 3316–3323
14. Skerrett, S. J., Liggitt, H. D., Hajjar, A. M., and Wilson, C. B. (2004) J. Immunol. 172, 3377–3381
15. Sasu, S., LaVerda, D., Qureshi, N., Golenbock, D. T., and Beasley, D. (2001) Circ. Res. 89, 244–250
16. Buhat, V., Faure, R., Thomas, L., Equils, O., and Arditi, M. (2001) J. Immunol. 167, 987–994
17. Netea, M. G., Kuilberg, B. J., Galama, J. M., Stalenhoef, A. F., Dinarello, C. A., and Van der Meer, J. W. (2002) Eur. J. Immunol. 32, 1186–1195
18. Rothfuchs, A. C., Trumstedt, C., Wiegell, H., and Rottenberg, M. E. (2004) J. Immunol. 172, 6345–6353
19. Peterson, E. M., Zhong, G. M., Carlson, E., and de la Maza, L. M. (1988) Infect. Immun. 56, 885–891
20. Ossewaarde, J. M., de Vries, A., Bestebroer, T., and Angulo, A. F. (1996) Appl. Environ. Microbiol. 62, 326–331
21. Peterson, R. M., de la Maza, L. M., Brade, L., and Brade, H. (1998) Infect. Immun. 66, 3848–3855
22. Pal, S., Lake, C. J., Barbour, A. G., Peterson, E. M., and de la Maza, L. M. (2003) Vaccine 21, 1455–1465
23. Mueller, M., Postius, S., Thimm, J. G., Guesnizius, K., Muehldorfer, L., and Hermann, C. (2004) Immunobiology 209, 599–608
24. Opitz, B., Puschel, A., Schneck, B., Hohe, A. C., Rosseau, S., Hammer, Schmidt, S., Schumann, R. R., Suttorp, N., and Hippenstiel, S. (2004) J. Biol. Chem. 279, 36426–36432
25. Rodriguez, N., Fend, F., Jennen, L., Schiemann, M., Wanta, N., Prazeres da Costa, C. U., Durr, S., Heinzmann, U., Wagner, H., and Metzke, T. (2005) J. Immunol. 174, 4836–4844
26. Rottenberg, M. E., Gigliotti Rothfuchs, A. C., Gigliotti, D., Svanholm, C., Bandholtz, L., and Wiegell, H. (1999) J. Immunol. 162, 2829–2836
27. Schnare, M., Barton, G. M., Holt, A. C., Takeda, K., Akira, S., and Medzhitov, R. (2001) Nat. Immunol. 2, 947–950
28. Zhou, S., Kunt-Jones, E. A., Mandell, L., Cerny, A., Chan, M., Golenbock, D. T., and Finberg, R. W. (2005) Eur. J. Immunol. 35, 822–830
29. Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., Mizutani, T., Shimada, N., Ohba, Y., Takaoka, A., Yoshida, N., and Taniguchi, T. (2005) Nature 434, 772–777