Development of a Secondary Immune Response to *Mycobacterium tuberculosis* Is Independent of Toll-Like Receptor 2

Amanda McBride, Kamlesh Bhatt, and Padmini Salgame*

University of Medicine and Dentistry of New Jersey, Department of Medicine, Center for Emerging Pathogens, Newark, New Jersey 07101

Published work indicates that the contribution of Toll-like receptor 2 (TLR2) to host resistance during acute *Mycobacterium tuberculosis* infection is marginal. However, in these studies, TLR2 participation in the memory immune response to *M. tuberculosis* was not determined. The substantial *in vitro* evidence that *M. tuberculosis* strongly triggers TLR2 on dendritic cells and macrophages to bring about either activation or inhibition of antigen-presenting cell (APC) functions, along with accumulating evidence that memory T cell development can be calibrated by TLR signals, led us to question the role of TLR2 in host resistance to secondary challenge with *M. tuberculosis*. To address this question, a memory immunity model was employed, and the response of TLR2-deficient (*TLR2 knockout [TLR2KO]*) mice following a secondary exposure to *M. tuberculosis* was compared to that of wild-type (WT) mice based on assessment of the bacterial burden, recall response, phenotype of recruited T cells, and granulomatous response. We found that upon rechallenge with *M. tuberculosis*, both WT and TLR2KO immune mice displayed similarly enhanced resistance to infection in comparison to their naïve counterparts. The frequencies of *M. tuberculosis*-specific gamma interferon (IFN-γ)-producing T cells, the phenotypes of recruited T cells, and the granulomatus responses were also similar between WT and TLR2KO immune mice. Together, the findings from this study indicate that TLR2 signaling does not influence memory immunity to *M. tuberculosis*.

*Corresponding author. Mailing address: University of Medicine and Dentistry of New Jersey, Department of Medicine, Center for Emerging Pathogens, Newark, NJ 07101. Phone: (973) 972-8647. Fax: (973) 972-0713. E-mail: salgampa@umdnj.edu.

†Published ahead of print on 20 December 2010.
proved or worsened the capacity of the host to generate memory immunity upon rechallenge with M. tuberculosis.

MATERIALS AND METHODS

Mice. C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). TLR2-deficient mice, originally developed by Takeuchi and colleagues (38), were bred and maintained under pathogen-free conditions at the transgenic-animal facility at the University of Medicine and Dentistry of New Jersey-New Jersey Medical School (UMDNJ-NJMS). M. tuberculosis-infected mice were housed in the biosafety level 3 (BSL3) facility at the Public Health Research Institute Center, UMDNJ-NJMS. The animal protocols used in this study were approved by the UMDNJ Institutional Animal Care and Use Committee.

Infection and immunization. The virulent Erdman strain of M. tuberculosis was used for all infections. Bacterial stocks were generated by initial passage in C57BL/6 mice. Bacterial colonies obtained from lung homogenates were grown in 7H9 medium until mid-log phase, and the culture was stored in aliquots at −80°C. Mice were infected via the respiratory route using a closed-air aerosolization system (In-Tox Products). The mice were exposed for 20 min to nebulized bacteria at a density optimized to deliver a dose of 50 to 100 CFU. The actual infection dose was determined by plating total lung homogenates from 2 mice at 24 h after aerosol exposure.

Establishment of memory immunity in M. tuberculosis-infected mice. In order to test the ability of hosts to generate a memory response in the absence of TLR2 signaling, a murine model of induction and development of memory response was established. Briefly, mice were aerosol infected with a low dose of M. tuberculosis (approximately 20 CFU). A group of uninfected wild-type (WT) and TLR2KO mice served as the control naïve mice. The bacterial infection was cleared by treating the mice for 12 weeks (starting at 5 weeks postinfection) with an antibiotic regimen of isoniazid (0.1 g/liter) and rifampin (0.15 g/liter) in their drinking water. Following drug cessation, the mice were rested for 10 weeks in order to allow complete development of the memory response. Midway through the rest period, whole lung and spleen homogenates from 4 mice were plated to ensure sterility, and no detectable CFU were found. Following the rest period, both naïve (control) and memory mice were challenged with M. tuberculosis via aerosol infection (approximately 50 CFU).

Determination of the bacterial burden. Lungs tissue was homogenized in phosphate-buffered saline (PBS) containing 0.05% Tween 80. The total number of CFU per lung was determined by plating 10-fold serial dilutions on Middlebrook 7H11 plates (Difco). CFU were counted after 21 days of incubation at 37°C.

Analysis of tissues. Lungs and draining mediastinal lymph node (MLNs) were harvested at the indicated time points postinfection. The right superior lobe of the lung was used for determining the bacterial burden. The right middle lobe was reserved for histological studies. The remaining lung tissue was perfused with 10 ml sterile distilled PBS (DPBS), cut into small pieces, and digested with 2 mg/ml collagenase D (Roche) for 30 min at 37°C. The digestion was stopped by adding 10 mM EDTA. The digested tissue was forced through a 40-μm strainer to obtain single-cell suspensions. Lymph node tissues were processed similarly, but without collagenase digestion. Red blood cells were lysed using ACK lysing buffer (Quality Biological, Inc.). The number of viable cells obtained from lungs and lymph nodes was determined by the trypan blue dye exclusion method. Similar procedures were used to determine the frequency of γδ T cells in lymph nodes.

Analysis of mediastinal lymph node gene expression levels. Gene expression levels from uninfected WT and TLR2KO mice were equivalent. A calibrator was total RNA from uninfected mediastinal lymph nodes. Baseline gene expression levels were set to 1. Gene expression levels in infected mice were normalized to the calibrator levels. Primer sequences used were as follows: IL-12p40, 5′-GAT GAC CCA GAT C-3′ and reverse, 5′-CAC AGC CTG GAT GGC TAC-3′; IL-10, 5′-TCC AAG ACC AAG GTG TCT AC-3′ and reverse, 5′-GGA GTC CAG ACT CAA TA-3′; and β-actin, forward, 5′-CCG GTA AAA GAT GAC CCA CAA GT-3′ and reverse, 5′-GAC AGC CTC GAT GCC TAC GT-3′. Relative gene expression was calculated as previously described (8). The calibrator was total RNA from uninfected mediastinal lymph nodes. Baseline gene expression levels were set to 1. Relative gene expression levels were measured using an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems). Data are presented as mean ± standard error of the mean (SEM).

RESULTS

TLR2KO mice control secondary M. tuberculosis infection like WT mice. The bacterial burdens in the lungs of immunized WT and TLR2KO mice, along with naïve WT and TLR2KO control mice, were compared. The numbers of viable bacilli were determined by plating serial dilutions of lung homogenates onto 7H11 agar plates. CFU were counted after 21 days of incubation at 37°C. Each time point includes 4 or 5 mice per group. The data are presented as mean CFU counts ± standard errors of the mean (SEM).

In order to test the ability of hosts to generate a memory response in the absence of TLR2 signaling, a murine model of induction and development of memory response was established. Briefly, mice were aerosol infected with a low dose of M. tuberculosis (approximately 20 CFU). A group of uninfected wild-type (WT) and TLR2KO mice served as the control naïve mice. The bacterial infection was cleared by treating the mice for 12 weeks (starting at 5 weeks postinfection) with an antibiotic regimen of isoniazid (0.1 g/liter) and rifampin (0.15 g/liter) in their drinking water. Following drug cessation, the mice were rested for 10 weeks in order to allow complete development of the memory response. Midway through the rest period, whole lung and spleen homogenates from 4 mice were plated to ensure sterility, and no detectable CFU were found. Following the rest period, both naïve (control) and memory mice were challenged with M. tuberculosis via aerosol infection (approximately 50 CFU).

Comparison of the bacterial burdens in WT and TLR2KO mice, along with naïve WT and TLR2KO control mice, were compared. The numbers of viable bacilli were determined by plating serial dilutions of lung homogenates onto 7H11 agar plates. CFU were counted after 21 days of incubation at 37°C. Each time point includes 4 or 5 mice per group. The data are presented as mean CFU counts ± standard errors of the mean (SEM).

RESULTS

TLR2KO mice control secondary M. tuberculosis infection like WT mice. The bacterial burdens in the lungs of immunized WT and TLR2KO memory mice and their naïve counterparts indicated that the M. tuberculosis burdens in the lungs at 2 weeks following infection were similar in the four groups of mice (Fig. 1). As reported previously (36), at 4 weeks postinfection, the bacterial burden in the lungs of WT naïve mice continued to increase while no further increase in CFU was noted in WT memory immune mice. The difference in lung bacterial burdens between the WT naïve and memory immune mice at this time was approximately 1 log unit. The TLR2KO memory immune mice were also able to control the bacterial burden at a significantly lower CFU than their naïve counterparts (P < 0.001).

Again as previously reported (36), at 8 weeks postinfection, the bacterial burden in the naïve WT mice had stabilized to numbers similar to those of their memory immune counterparts. A similar trend was observed in the TLR2KO mice. These data indicate that the generation of memory immunity involved in the rapid control of bacterial replication in the
lungs upon secondary challenge is independent of the presence of TLR2 signals at the time of priming.

**Activation profile of lung CD4 and CD8 T cells following challenge with *M. tuberculosis***. We next characterized the lung cellular infiltrates of naïve and memory mice at 2 and 4 weeks following challenge with *M. tuberculosis* (Fig. 2). Flow cytometric analysis showed that at 2 weeks postinfection the total cell accumulation (Fig. 2A) and the numbers of CD4 (Fig. 2B) and CD8 (Fig. 2C) T cells present in the lungs were not significantly different between WT and TLR2KO naïve and memory mice. At 4 weeks, total cell recruitment (Fig. 2A) and accumulation of CD4 (Fig. 2B) and CD8 (Fig. 2C) T cells were higher in both groups of naïve mice than in their memory counterparts, although this increase was only significant in the TLR2KO mice. Upon activation, T cells upregulate cell surface expression of CD44 and concomitantly downregulate expression of CD62L. We next determined the activation status of CD4 and CD8 T cells present in the lungs of the four groups of mice and found that there was a modest, albeit not statistically significant (in TLR2KO mice), increase in the percentage of CD4 T cells with the activated phenotype (CD44hi CD62Llo) in both groups of memory mice in comparison to their naïve counterparts. By 4 weeks postinfection, the percentages of activated CD4 cells were equivalent in all four groups of mice (Fig. 2D). In the CD8 population, the percentages of activated cells were similar between naïve and memory mice at 2 weeks. At 4 weeks, all 4 groups of mice exhibited a further increase in the percentage of activated CD8 cells in the lungs; however, the WT memory group did not show the same level of activation as its naïve counterpart (Fig. 2E).

**T cell recall responses are not altered in the absence of TLR2**. Consistent with the increase in activated T cells, a high frequency of cells secreting IFN-γ was also present in the lungs of memory immune WT and TLR2KO mice at 2 weeks postinfection, while these cells were barely detectable at this time point in the naïve groups (Fig. 3). At week 4 postinfection, all four groups exhibited an increase in IFN-γ-secreting cells, and there was no statistically significant difference between the four groups.

---

**FIG. 2.** Characterization of T cells infiltrating the lungs of naïve and memory immune mice. Lungs were harvested from naïve (N) and memory immune (M) mice, and single-cell suspensions were prepared at 2 and 4 weeks after *M. tuberculosis* challenge. (A) The number of viable cells in the lungs was determined by the trypan blue exclusion method. Lung cells (1 × 10⁶) were stained with antibodies against CD4, CD8, CD44, and CD62L and analyzed by flow cytometry. Lymphocytes were gated on first, followed by further gating on CD4 and CD8 populations. (B and C) The absolute numbers of CD4 (B) and CD8 (C) T cells in the lungs were determined by calculating the percentage of gated cells multiplied by the total lung cell number. (D and E) To determine the activation status of the T cell populations, further gating on CD44hi CD62Llo cells in the CD4-gated population (D) and in the CD8-gated population (E) was performed. Each time point includes 4 or 5 mice per group, and the data are presented as mean counts and SEM. **, *P < 0.01; ***, *P < 0.001.
The findings so far indicate that, although total cell numbers present in the lungs are similar between naïve and memory mice at 2 weeks postinfection, memory immune mice have increased numbers of activated T cells capable of secreting IFN-γ. The findings also indicate that the TLR2KO memory immune mice, like WT mice, can recruit antigen-specific T cells rapidly to the lung.

**Granulomatous response.** The memory response to *M. tuberculosis* leads to faster control of bacterial growth, which can be visualized histopathologically as a decrease in the size of the lung granulomatous lesions (19). Consistent with the reduction in CFU observed at 4 weeks postinfection, both WT and TLR2KO memory mice presented with smaller granulomas that were more lymphocytic in nature than those of their naïve counterparts (Fig. 4A). While the granulomatous inflammation was still localized in both WT and TLR2KO naïve mice, the lesions in naïve mice were larger and consisted mainly of macrophages and scattered lymphocytes. Measurement of granuloma size demonstrated significant differences between the naïve and memory groups; however, there were no differences between WT and TLR2KO mice in either group (Fig. 4B). Overall, these observations demonstrate that in the presence or absence of TLR2, memory immunity results in similar early containment of bacteria and control of inflammation in the lungs.

**The cytokine milieu in lymph nodes is not affected by the absence of TLR2.** To determine if absence of TLR2 signaling altered the cytokine environment during naïve T cell priming, we performed a comparative evaluation of IL-10 and IL-12p40 gene expression in mediastinal lymph node cells at 2 and 3 weeks postinfection (Fig. 5). At 2 weeks, neither WT nor TLR2KO mice had increased expression of IL-10, while both groups showed a moderate increase in IL-12p40. At 3 weeks, expression of both cytokines was moderately increased in WT and TLR2KO mice. All changes in cytokine expression were equivalent between WT and TLR2KO mice, indicating that TLR2 signals do not affect the cytokine milieu in the draining lymph nodes during these early stages of naïve T cell priming.

**DISCUSSION**

The contribution of TLR2 to host immunity has been studied primarily during acute infection, and the collective data indicate that TLR2-deficient mice exhibit minimal (1, 7) to no (33, 34, 37) decrease in acute resistance to low-dose infection with *M. tuberculosis*. Since TLR2 triggering induces manifold opposing changes to APC functions and to the cytokine milieu generated during T cell priming, our goal was to examine the consequence of TLR2 deficiency for the expression of memory immunity.
Consistent with previous studies, we found that primary infection of mice with *M. tuberculosis* and subsequent drug treatment led to the generation of a pool of memory T cells that upon secondary challenge expanded rapidly to control bacterial growth in the lungs and to hold it at a constant level. Interestingly, despite *in vitro* evidence that TLR2 controls several aspects of antigen presentation and cytokine production functions of APC (reviewed in reference 13), absence of TLR2 during *in vivo* infection did not affect memory immunity. Contrary to predictions, we found that memory immunity that developed in the host in the absence of TLR2 did not result in better control of *M. tuberculosis*. The removal of TLR2 also did not impair the characteristic early stabilization of the secondary infection. Studies have indicated that TLR2-MyD88 signaling on CD8 T cells is critical for clonal expansion and memory formation following vaccinia virus infection (32). However, such a requirement for TLR2 on either CD4 or CD8 T cells was not necessary for memory T cell responses following *M. tuberculosis* infection. Equivalent numbers of antigen-specific memory effector T cells were generated in both WT and TLR2KO mice following secondary challenge.

The findings presented here suggest that following *M. tuberculosis* infection, TLR2 may not participate in the early innate immune response that regulates naïve T cell differentiation into effector and memory T cells. Consistent with this, we did not see any significant differences in the expression of IL-12 and IL-10 in the draining lymph nodes of 2- and 3-week-infected wild-type and TLR2KO mice. Since host resistance to acute *M. tuberculosis* infection is moderately compromised in mice deficient in TLR9 (1), it is possible that TLR9 may contribute to early innate responses to *M. tuberculosis* infection and regulate both effector and memory T cell development.

MyD88-dependent resistance to *M. tuberculosis* infection is complex (reviewed in reference 20) and involves both TLR- (1) and IL-1 receptor 1 (IL-1R1)-mediated signals (9, 23). Mice deficient in MyD88 are highly susceptible to acute *M. tuberculosis* infection (10, 14, 35) but nonetheless are able to induce *M. tuberculosis*-specific adaptive immune responses (10, 14). Consistent with their ability to generate adaptive immunity, *Mycobacterium bovis* BCG immunization can protect MyD88-deficient mice from acute *M. tuberculosis* infection (10). Therefore, it is quite likely that signals other than TLR/MyD88 regulate induction of memory immunity during *M. tuberculosis* infection.

We propose that TLR2 signaling may be essential for cytokine production within the granuloma. Macrophages infected *in vitro* release mycobacterial lipids (3), so it is conceivable that TLR2 ligands are unmasked and secreted during *in vivo* infection within the granulomas. The secreted ligands can influence activation of uninfected macrophages and other cell types present in the lung. Thus, we posit that TLR2's contribution to host resistance is at the level of granuloma maintenance, is manifested later in infection, and is thereby temporally segregated from TLR9 functions. Consistent with this, the greatest effect on the progression of tuberculosis disease is seen in mice doubly deficient in TLR2 and TLR9 (1).

The risk of developing TB has been shown to be associated with polymorphisms within the TLR2 gene (25, 39), and shorter guanine-thymine (GT) repeat polymorphism in intron II of the TLR2 gene were more common in TB patients and correlated with lower expression of TLR2 (42). The mechanisms through which these TLR2 polymorphisms affect host defense remain unclear. Our findings suggest that it is perhaps at the level of macrophage effector functions in the granuloma and not at the level of induction of effector or memory Th1 immunity. This possibility is supported by the fact that infection of TLR2 mice with a high dose leads to inflammation and enhanced bacterial burden in the lungs (34). Ongoing studies in the laboratory are investigating the molecular signals downstream of TLR2 that contribute to the maintenance of the tubercle granuloma.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant AI071844 to P.S. and F30HL094028 to A.M. We thank David Lagunaoff and Luke Fritzky for help with the histopathological evaluations.

REFERENCES

1. Bafica, A., et al. 2005. TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis. J. Exp. Med. 202:1715–1724.
2. Banaiee, N., E. Z. Kincaid, U. Buchwald, W. R. Jacobs, Jr., and J. D. Ernst, 2000. Prophylactic inhibition of macrophage responses to IFN-gamma by live virulent Mycobacterium tuberculosis is independent of mature mycobacterial lipoproteins but dependent on TLR2. J. Immunol. 167:3019–3027.
3. Beatty, W. L., and D. G. Russell, 2000. Identification of mycobacterial surface proteins released into subcellular compartments of infected macrophages. Infect. Immun. 68:6997–7002.
4. Bhatt, K., et al. 2009. B7 costimulation is critical for host control of chronic Mycobacterium tuberculosis infection. J. Immunol. 182:3793–3800.
5. Brightbill, H. D., et al. 1999. Host defense mechanisms triggered by microbial lipoproteins through Toll-like receptors. Science 286:732–736.
6. Cooper, A. M., J. E. Callahan, M. Keen, J. T. Belisle, and I. M. Orme, 1997. Expression of memory immunity in the lung following re-exposure to Mycobacterium tuberculosis. Tuber. Lung Dis. 78:67–73.
7. Drennan, M. R., et al. 2004. Toll-like receptor 2-deficient mice succumb to Mycobacterium tuberculosis infection. Am. J. Pathol. 164:49–57.
8. Fortune, S. M., et al. 2004. Mycobacterium tuberculosis inhibits macrophage responses to IFN-gamma through myeloid differentiation factor 88-dependent and -independent mechanisms. J. Immunol. 172:6272–6280.
9. Fremond, C. M., et al. 2007. IL-1 receptor-mediated signal is an essential component of MyD88-dependent innate response to Mycobacterium tuberculosis infection. J. Immunol. 179:1178–1189.
10. Fremond, C. M., et al. 2004. Fatal Mycobacterium tuberculosis infection despite adaptive immune response in the absence of MyD88. J. Clin. Invest. 114:1790–1799.
11. Gehring, A. J., K. M. Dobos, J. T. Belisle, C. V. Harding, and W. H. Boom. 2004. Mycobacterium tuberculosis LprG (Rv1411c): a novel TLR2 ligand that inhibits human macrophage class II MHc antigen processing. J. Immunol. 173:2660–2668.
12. Gillieron, M., et al. 1997. Mycobacterium smegmatis phosphonoformates-glucose-6-1-phosphonoformates. Structure and localization of alkali-labile and alkaline stable phosphonoformates. J. Biol. Chem. 272:117–124.
13. Harding, C. V., and W. H. Boom, 2010. Regulation of antigen presentation by Mycobacterium tuberculosis: a role for Toll-like receptors. Nat. Rev. Microbiol. 8:296–307.
14. Holscher, C., et al. 2008. Containment of aerogenic Mycobacterium tuberculosis infection in mice does not require MyD88 adaptor function for TLR2, -4 and -9. Eur. J. Immunol. 38:680–694.
15. Jiang, S., S. Uematsu, S. Akira, and P. Salgame, 2004. IL-6 and IL-10 induction from dendritic cells in response to Mycobacterium tuberculosis is predominantly dependent on TLR2-mediated recognition. J. Immunol. 173:3392–3397.
16. Jones, B. W., et al. 2001. Different Toll-like receptor agonists induce distinct macrophage responses. J. Leukoc. Biol. 69:1036–1044.
17. Joshi, N. S., et al. 2007. Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription regulator. Immunity 27:303–315.
18. Kincaid, E. Z., et al. 2007. Codominance of TLR2-dependent and TLR2-independent modulation of MHC class II in Mycobacterium tuberculosis infection in vivo. J. Immunol. 179:3187–3195.
19. Kipnis, A., S. Irwin, A. A. Izzo, R. J. Basaraba, and I. M. Orme, 2005. Memory T lymphocytes generated by Mycobacterium bovis BCG vaccination reside within a CD4(+)CD62 ligandhi population. Infect. Immun. 73:7759–7764.
20. Korbel, D. S., B. E. Schneider, and U. E. Schäible. 2008. Innate immunity in tuberculosis: myths and truth. Microbes Infect. 10:995–1004.
21. Lazarevic, V., D. J. Vankura, S. J. DiVito, and J. L. Flynn. 2005. Induction of Mycobacterium tuberculosis-specific primary and secondary T-cell responses in interleukin-15-deficient mice. Infect. Immun. 73:2910–2922.
22. Liu, P. T., et al. 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311:1770–1773.
23. Mayer-Barber, K. D., et al. Caspase-1 independent IL-1beta production is critical for host resistance to Mycobacterium tuberculosis and does not require TLR signaling in vivo. J. Immunol. 184:3326–3330.
24. Noss, E. H., et al. 2001. Toll-like receptor 2-dependent inhibition of macrophage class II MHC expression and antigen processing by 19-kDa lipoprotein of Mycobacterium tuberculosis. J. Immunol. 167:910–918.
25. Ogus, A. C., et al. 2004. The Arg753Gln polymorphism of the human Toll-like receptor 2 gene in tuberculosis disease. Eur. Respir. J. 23:219–223.
26. Pai, R. K., M. Convery, T. A. Hamilton, W. H. Boom, and C. V. Harding. 2003. Inhibition of IFN-gamma-induced class II transactivator expression by a 19-kDa lipoprotein from Mycobacterium tuberculosis: a potential mechanism for immune evasion. J. Immunol. 171:175–184.
27. Pai, R. K., et al. 2004. Prolonged Toll-like receptor signaling by Mycobacterium tuberculosis and its 19-kilodalton lipoprotein inhibits gamma interferon-induced regulation of selected genes in macrophages. Infect. Immun. 72:6603–6614.
28. Pearce, E. L., and H. Shen. 2007. Generation of CD8 T cell memory is regulated by IL-12. J. Immunol. 178:2074–2081.
29. Pecora, N. D., A. J. Gehring, D. H. Canaday, W. H. Boom, and C. V. Harding. 2006. Mycobacterium tuberculosis LprA is a lipoprotein agonist of TLR2 that regulates innate immunity and APC function. J. Immunol. 177:422–429.
30. Pompei, L., et al. 2007. Disparity in IL-12 release in dendritic cells and macrophages in response to Mycobacterium tuberculosis is due to use of distinct TLRs. J. Immunol. 178:5192–5199.
31. Quesniaux, V. J., et al. 2004. Toll-like receptor 2 (TLR2)-dependent-positive and TLR2-independent-negative regulation of proinflammatory cytokines by mycobacterial lipomannans. J. Immunol. 172:4425–4434.
32. Quigley, M., J. Martinez, X. Huang, and Y. Yang. 2008. A critical role for direct TLR2-MD8 signaling in CD8 T cell clonal expansion and memory formation following vaccinia viral infection. Blood 111:2256–2264.
33. Reiling, N., S. Ehlers, and C. Holscher. 2008. MyD88 and un-TOLLed truths: sensor, instructive and effector immunity to tuberculosis. Immunol. Lett. 116:15–23.
34. Reiling, N., et al. 2002. Cutting edge: Toll-like receptor (TLR)2- and TLR4-mediated pathogen recognition in resistance to airborne infection with Mycobacterium tuberculosis. J. Immunol. 169:3480–3484.
35. Scanga, C. A., et al. 2004. 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. 72:2400–2404.
36. Serbina, N. V., and J. L. Flynn. 2001. CD8(+) T cells participate in the memory immune response to Mycobacterium tuberculosis infection. Infect. Immun. 69:4320–4328.
37. Sugawara, I., et al. 2003. Mycobacterial infection in TLR2 and TLR4 knock-out mice. Microbiol. Immunol. 47:327–336.
38. Takeuchi, O., et al. 1999. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 11:443–451.
39. Velez, D. R., et al. Variants in Toll-like receptors 2 and 9 influence susceptibility to pulmonary tuberculosis in Caucasians, African-Americans, and West Africans. Hum. Genet. 127:65–73.
40. Verver, S., et al. 2005. Rate of reinfection tuberculosis after successful treatment is higher than rate of new tuberculosis. Am. J. Respir. Crit. Care Med. 171:1430–1435.
41. Wilson, D. C., et al. 2004. Differential regulation of effector- and central-memory responses in Toxoplasma gondii infection by IL-12 revealed by tracking of Tgd057-specific CD8+ T cells. PLoS Pathog. 6:e1000815.
42. Yin, J. J., et al. 2004. A microsatellite polymorphism in intron 2 of human Toll-like receptor 2 gene: functional implications and racial differences. FEMS Immunol. Med. Microbiol. 40:163–169.

Editor: J. L. Flynn