Heat-Killed Brucella abortus Induces TNF and IL-12p40 by Distinct MyD88-Dependent Pathways: TNF, Unlike IL-12p40 Secretion, Is Toll-Like Receptor 2 Dependent

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J Immunol 2003; 171:1441-1446; doi: 10.4049/jimmunol.171.3.1441
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Heat-Killed Brucella abortus Induces TNF and IL-12p40 by Distinct MyD88-Dependent Pathways: TNF, Unlike IL-12p40 Secretion, Is Toll-Like Receptor 2 Dependent

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Cattle and humans are susceptible to infection with the Gram-negative intracellular bacterium Brucella abortus. Heat-killed B. abortus (HKBA) is a strong Th1 adjuvant and carrier. Previously, we have demonstrated that dendritic cells produce IL-12 in response to HKBA stimulation. In the present study, we use knockout mice and in vitro reconstitution assays to examine the contribution of signaling by Toll-like receptors (TLRs) and their immediate downstream signaling initiator, myeloid differentiation protein MyD88, in the activation following stimulation by HKBA. Our results show that HKBA-mediated induction of IL-12p40 and TNF is dependent on the adapter molecule MyD88. To identify the TLR involved in HKBA recognition, we examined HKBA responses in TLR2- and TLR4-deficient animals. TNF responses to HKBA were TLR4 independent; however, the response in TLR2-deficient mice was significantly delayed and reduced, although not completely abolished. Studies using Chinese hamster ovary/CD14 reporter cell lines stably transfected with either human TLR2 or human TLR4 confirmed the results seen with knockout mice, namely TLR2, but not TLR4, can mediate cellular activation by HKBA. In addition, human embryonic kidney 293 cells, which do not respond to HKBA, were made responsive by transfecting TLR2, but not TLR4 or TLR9. Taken together, our data demonstrate that MyD88-dependent pathways are crucial for activation by HKBA and that TLR2 plays a role in TNF, but not IL-12p40 pathways activated by this microbial product. The Journal of Immunology, 2003, 171: 1441–1446.

Microbial recognition by host molecules can be mediated by several different host cell membrane receptors. Toll-like receptors (TLR)2 constitute a family of membrane molecules that have been identified as receptors for bacteria and bacterial products transducing signals via a common adaptor molecule, myeloid differentiation protein MyD88, and, consequently, leading to host cell activation of proinflammatory responses (1–4). Activation of MyD88-dependent pathways results in NF-κB translocation to the nucleus. This pathway is believed to be a major component of the signaling cascade triggered by these receptors; however, other pathways of induction of gene expression have recently been described involving adapter molecules such as TIRAP/Mal and TRIF (5–8).

Activation of the immune system by heat-killed Brucella abortus (HKBA) has been shown to potently activate the innate as well as the adaptive immune systems, leading to a proinflammatory response that favors the differentiation of T cell responses toward a Th1 profile (9–11).

We have taken advantage of B. abortus as an immune delivery system for Ags, including those derived from HIV-1 (12, 13). In HIV-1 infection, B. abortus may be particularly beneficial, because it is capable of inducing Ab and CTL responses in the absence of T cell help (13, 14).

Receptor usage in the innate immune system by heat-killed B. abortus is unknown. In this study, we show that HKBA can stimulate cells via MyD88 and acts on TLR2, resulting in NF-κB translocation. Secretion of proinflammatory cytokines, such as TNF and IL-12, was shown to involve MyD88. Interestingly, despite the fact that the induction of both cytokines was dependent on an intact MyD88-dependent cascade, the requirement for TLRs was different. We found that TNF induction was dependent on TLR2; however, IL-12 secretion was unaltered in TLR2−/− mice. Taken together, the results presented in this work suggest that a single pathogen may use several TLR pathways, resulting in a complex triggering of proinflammatory responses that probably impacts on the nature and the intensity of the ensuing immune response.

Materials and Methods

Mice and in vivo stimulation

C57BL/6, C3H/HeJ, and C3H/HeOuJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME). MyD88−/−, TLR2−/−, and TLR4−/− mice were provided by S. Akira (Osaka University, Osaka, Japan) (15–17). These animals were bred and maintained at an American Association of Laboratory Animals animal facility (18). Animals were used according to National Institutes of Health guidelines on animal use and care.

For in vivo stimulations, mice were injected i.p. with HKBA (106 organisms) or PBS. After injection, at the indicated time point, sera were collected and stored at −80°C until assayed. In each in vivo experiment, at least three mice were used per stimulation and repeated at least twice. The SD represents variability between individual mice.

Reagents

HKBA was kindly provided by B. Martin (United States Department of Agriculture, Ames, IA) and was washed extensively with PBS before use.

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Received for publication February 14, 2003. Accepted for publication May 28, 2003.

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2 Abbreviations used in this paper: TLR, Toll-like receptor; CHO, Chinese hamster ovary; DC, dendritic cell; HEK, human embryonic kidney; HKBA, heat-killed B. abortus; MyD88, myeloid differentiation protein; ODN, oligodeoxynucleotide; PGN, peptidoglycan; RLUs, relative light units; WT, wild type.

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0022-1767/03/$02.00
B. abortus LPS was purified by butanol extraction, as described previously (19). LPS derived from Escherichia coli and polymyxin B sulfate were purchased from Sigma-Aldrich (St. Louis, MO). Peptidoglycan (PGN) from Staphylococcus aureus was from Fluka (Sigma-Aldrich). CpG oligodeoxynucleotides (ODN) were purchased from Life Technologies (Rockville, MD) (20).

Cell isolation and in vitro stimulation

Spleen cell suspensions were prepared by Liberase CI (Roach Biochemicals, Indianapolis, IN) digestion, and processed as previously described (21).

Enriched dendritic cells (DC) were obtained by labeling splenocytes with anti-CD11c MACS beads (Miltenyi Biotec, Auburn, CA) and passed through a MACS selection column (Miltenyi Biotec). The cells purified from the column were routinely 70–85% CD11c+ as determined by flow cytometry (22).

For in vitro stimulation, splenocytes or enriched DC were cultured in complete RPMI medium at a concentration of 105 cells/ml or 106 cells/ml, respectively. Cultures were incubated in the presence or absence of different stimuli in RPMI 1640 (Quality Biological, Gaithersburg, MD) supplemented with 10% heat-inactivated FBS, penicillin-streptomycin, HEPES buffer, 2-ME, nonessential amino acids, pyruvate, and glutamine. Following stimulation, culture supernatants were collected after overnight incubation and stored at −20°C until assayed.

Chinese hamster ovary (CHO) cell lines

The CHO reporter cell lines (CHO/CD14, CHO/CD14/TLR2, and CHO/CD14/TLR4) (23) were maintained as adherent monolayers in Ham’s F-12 medium (BioWhittaker, Walkersville, MD) with 10% low endotoxin FBS (HyClone, Logan, UT) and antibiotics at 37°C, 5% CO2. All of the cell lines are derived from CHO/CD14, known as clone 3E10; the engineering of this clone has been previously described in detail (24). This cell line expresses the human TLR, 6 ng pEF-boss-hMD2 (for TLR4), and control plasmid for a CD25 expression vector. This promoter contains a NF-κB binding site; CD25 expression is completely dependent upon NF-κB translocation to the cell nucleus. Cells expressing TLRs were constructed by stable transfection of the CHO/CD14 reporter cell line with the cDNA for human TLR2 or TLR4, as described previously (23).

Flow cytometry analysis

CHO reporter cells were plated in 12-well tissue culture dishes at a density of 3 × 104 per well. After overnight incubation, various microbial stimuli were added as indicated, in a total volume of 0.5 ml of medium/well. Following incubation for 18 h, the cells were harvested with Trypsin-Versene (Biofluids, Rockville, MD), washed with PBS, stained with PE-labeled anti-CD25 (Caltag Laboratories, Burlingame, CA), and examined by flow cytometry (BD Biosciences, San Jose, CA) (23). Analyses were performed using CellQuest software (BD Biosciences).

Transient transfection and reporter assay

Human embryonic kidney (HEK) 293 cells were seeded at 104 cells/well and transfected for 3 h in 96-well plates by CaPO4 precipitation method with 12 ng IgκB luciferase, 12 ng pSvβ-galactosidase, 2.5 ng pcDNA human TLR, 6 ng pEF-boss-hMD2 (for TLR4), and control plasmid for a total of 100 ng. After overnight culture, the cells were treated with various stimuli for 24 h. Cell lysates were assayed for luciferase activity using the Luciferase Reporter Assay System (Promega, Madison, WI) and for β-galactosidase activity (Tropix, Applied Biosystems, Foster City, CA). Luciferase activity of individual transfections was normalized against β-galactosidase activity. Data reflect the luciferase relative light units (RLU) divided by the control β-galactosidase RLU.

Quantification of cytokines

Level of secreted cytokines in cell culture medium and mouse sera was measured using ELISA technology. For mouse TNF (previously known as TNF-α), commercial kits from R&D Systems (Minneapolis, MN) and Pierce-Endogen (Woburn, MA) were used, while for IL-12p70, either commercial kits from Pierce-Endogen or a previously described ELISA method (22) were used. All samples were assayed in duplicates or triplicates and repeated in separate experiments at least twice.

Statistical analysis

Results were expressed as the mean ± SD. Data were analyzed, as appropriate, by unpaired t tests or one-way ANOVA using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA). Differences were considered statistically significant at p < 0.05.

Results

HKBA stimulation of TNF in vivo and in vitro in mice is MyD88 and TLR2 dependent

The effect of HKBA on TNF was studied in vivo and in vitro in wild-type (WT) mice and mice with defects in MyD88, TLR2, or TLR4 expression. Following injection of HKBA i.p. into WT mice, TNF was induced and serum levels peaked at 2 h. In contrast, HKBA failed to elicit TNF at the different time points in MyD88−/− mice (Fig. 1A). This indicates that TNF induction by HKBA is dependent on MyD88 and therefore likely to use a TLR.

To determine whether TLRs are involved in HKBA up-regulation of TNF expression, different mice were studied that have mutant or targeted knockout of specific TLR genes. In Fig. 1B, C3H/HeJ mice expressing a mutant version of TLR4 failed to respond to E. coli LPS as expected (25, 26). In contrast, induction of TNF by HKBA in C3H/HeJ was not reduced compared with the nonmutant C3H/HeOuJ strain. Interestingly, the kinetics of LPS and HKBA induction of TNF was different in that the LPS response reached a maximum before the HKBA. This suggests that two distinct pathways are involved in stimulation of TNF by LPS and HKBA. However, when TLR2−/− mice were studied, it became apparent that the early (1- to 3-h) TNF response to HKBA was dependent on TLR2. There was a delayed response to TNF in the TLR2−/− mice, not seen in the TLR2+/+ counterparts (Fig. 1A). This suggests that TNF secretion in vivo following HKBA occurs...
via two pathways, only one of which, the early activation, is MyD88/TLR2 dependent. Because the delayed response was not observed in MyD88−/− mice (Fig. 1A), it is MyD88 dependent, but TLR2 independent.

These results were confirmed by in vitro experiments. As expected, PGN stimulated TNF secretion from WT splenocytes, but not from splenocytes obtained from MyD88−/− or TLR2−/− mice (Fig. 2). Similarly, addition of HKBA to WT murine splenocytes resulted in TNF secretion within 24 h of culture. However, when HKBA was added to splenocytes from MyD88−/− and TLR2−/− mice, the response within 24 h was markedly reduced (Fig. 2). This is in accordance with the early induction of TNF in vivo by HKBA, which was also mediated by a MyD88/TLR2-dependent pathway. As shown in Fig. 3, LPS derived from E. coli and B. abortus act via TLR4 because they fail to induce TNF from TLR4-mutant mice (C3H/HeJ). In contrast to these LPS preparations, HKBA was able to elicit TNF secretion from splenocytes (Fig. 3) and peritoneal macrophages (data not shown) from TLR4-mutant C3H/HeJ mice, indicating that HKBA activation is TLR4 independent.

Ectopic expression of TLRs reveals that HKBA activates NF-κB in a TLR2-dependent manner

CHO/CD14 cells were stably transfected with human CD25 under the regulation of NF-κB. Because the CHO reporter cells express hamster CD14 and low levels of hamster TLR4, they respond to E. coli LPS (which acts mainly on TLR4) by up-regulating CD25 independently of whether they are transfected with an appropriate TLR or not (Fig. 4A) (23). As previously observed, E. coli LPS activation is inhibitable by polymyxin B, an antibiotic that binds and neutralizes LPS. Stimulation by PGN was confirmed to activate NF-κB via TLR2 regardless of addition of polymyxin B (Fig. 4B), as previously observed (16, 27, 28). Similarly, HKBA (Fig. 4C) activated via TLR2 only and was not inhibitable by polymyxin B. This up-regulation of CD25 expression in response to HKBA is dose dependent (Fig. 4D). Thus, TLR2 activation by HKBA in either murine or human cells is analogous in that they both involve translocation of NF-κB to the nucleus.

In an attempt to further characterize the interaction of HKBA with TLR2, we performed experiments in which HEK 293 cells were transiently transfected with human TLR2, TLR4 plus MD2, or TLR9. Luciferase was used as a reporter gene for NF-κB activation. As shown in Fig. 5, HKBA activated cells expressing TLR2, but not cells expressing TLR4 or TLR9. In contrast, E. coli LPS and CpG ODN were able to activate via TLR4 and TLR9, respectively (17, 29). PGN-induced NF-κB activation is mediated by TLR2, as previously reported (27).

**Induction of IL-12 p40 secretion by HKBA is MyD88 dependent, but TLR2/4 independent**

IL-12 production is induced mainly in DC after HKBA stimulation (30). Therefore, we decided to investigate whether HKBA-induced IL-12 responses follow the same receptor requirements as TNF and NF-κB responses. To answer this question, we studied the expression of this cytokine in vivo and in vitro following HKBA stimulation in WT, as well as MyD88−/−, TLR2−/−, and TLR4-deficient mice. Time course experiments in vivo showed that IL-12 p40 can be detected in serum following HKBA injection in WT, TLR2−/−, and TLR4-mutant mice (C3H/HeJ), but not in MyD88−/− mice (Fig. 6, A and B).

In vitro experiments were performed to further confirm this finding using splenic DC, because DC are the major source of IL-12 secretion following HKBA stimulation (30). Following stimulation with HKBA, in vitro DC secretion of IL-12 p40 was abrogated in cells from MyD88−/− mice (data not shown). This was not the case in WT, TLR2−/−, or TLR4−/− mice (Fig. 7A). This is in agreement with the in vivo experiments and implies that, like TNF, IL-12 p40 secretion is dependent on MyD88, but unlike TNF, IL-12 p40 secretion following activation by HKBA is not dependent on TLR2.

To determine whether the different TLR usage of HKBA resulting in TNF and IL-12 p40 secretion was occurring in the same APC preparation, enriched splenic DC were assessed. As seen in Fig. 7A, HKBA stimulation induced IL-12 p40 secretion from enriched DC in all the mouse strains tested, indicating that HKBA elicited IL-12 p40 in a TLR2/4-independent manner. In contrast, IL-12 p40 secretion following PGN was TLR2 dependent, and E. coli LPS was TLR4 dependent, respectively. Unlike IL-12 p40, TNF induction by HKBA was TLR2 dependent because TNF secretion from DC following HKBA was observed in WT and TLR4−/− mice, but not in TLR2−/− mice (Fig. 7B). Similar to IL-12 p40 secretion, TNF secretion by PGN was TLR2 dependent, and E. coli LPS was TLR4 dependent, respectively. This indicates that HKBA stimulates IL-12 p40 and TNF in enriched DCs via different pathways. This experiment was repeated with peritoneal macrophages (data not shown), and it was shown that, like DC, HKBA induction of IL-12 p40 is TLR2 independent, whereas TNF induction is TLR2 dependent.

![FIGURE 2. HKBA induction of TNF secretion in vitro is MyD88 and TLR2 dependent. Splenocytes from C57BL/6, TLR2−/−, and MyD88−/− mice were cultured in the presence of medium, HKBA (10⁸ organisms/ml), and PGN (10 μg/ml), which is known to activate via TLR2. Supernatants were collected after 18 h and assayed for TNF by ELISA. *p < 0.05, compared with WT mice. Data are representative of three similar experiments.](http://www.jimmunol.org/)

![FIGURE 3. HKBA induction of TNF secretion in vitro is TLR4 independent. Splenocytes from C3H/HeOuJ and C3H/HeJ (TLR4-mutant) mice were cultured in the presence of medium, HKBA (10⁸ organisms/ml), E. coli LPS (30 μg/ml), and B. abortus LPS (30 μg/ml). Supernatants were collected after 18 h and assayed for TNF by ELISA. Data are representative of two similar experiments.](http://www.jimmunol.org/)
MyD88 is a common adapter molecule for most known TLRs and for IL-1R and IL-18R (15). Thus, the fact that IL-12p40 secretion did require MyD88, but not TLR2 or TLR4, implies that other TLRs are involved or that IL-1R and/or IL-18R are involved. In a separate set of experiments (data not shown), enriched murine DC were stimulated with HKBA in the presence or absence of Abs, which block IL-1 and IL-18. Additionally, caspase peptide was also used to block the cleavage of pro-IL-1 and IL-18. Inhibition of the IL-1 and IL-18 pathways had no effect on the quantity of IL-12p40 secreted in response to HKBA (data not shown). Taken together, these results suggest that IL-12p40 secretion following HKBA stimulation is dependent on a receptor that uses MyD88, but that this receptor is different from TLR2, 4, or 9, and that IL-1R and IL-18R are not involved.

Discussion

B. abortus behaves as a Th1-like activator of the immune systems of humans and mice (9, 31). We have shown that it can be used as an effective carrier for HIV-1 peptides and proteins to induce long-term Ab and CTL responses systemically and mucosally in mice and monkeys (13). B. abortus also induces β-chemokine secretion from human CD8+ T cells and macrophages (32). Additionally,

FIGURE 4. HKBA induces CD25 expression via NF-κB in CHO cells stably transfected with TLR2, but not TLR4. CHO reporter cells that express CD14 and TLR4 were stably transfected with CD25, whose promoter is under the control of NF-κB. Some of these cells were cotransfected with either human TLR4 or TLR2. Following stimulation with medium (shaded), in the presence of polymyxin B (PMX, 10 μg/ml) (dotted line) and by: A, E. coli LPS (100 ng/ml); B, PGN (5–10 μg/ml); C, HKBA (10⁶ organisms/ml), all in solid lines; and D, HKBA, 10⁷ (dotted line), 5 × 10⁷ (thin solid line), and 10⁸ (thick solid line). These cells were subjected to flow cytometry to assess CD25 expression. Data are representative of four (A–C) and two (D) similar experiments.

FIGURE 5. HKBA induces luciferase activity under control of NF-κB in HEK 293 cells transiently transfected with TLR2, but not TLR4 or TLR9. HEK 293 cells were transiently transfected with β-galactosidase and luciferase under control of NF-κB, and either human TLR2, TLR4 plus MD2, or TLR9. They were stimulated in vitro with either HKBA (10⁸/ml), PGN (10 μg/ml), E. coli LPS (100 ng/ml), or CpG ODN (1 μM). The relative luciferase activity, as a measure of NF-κB activity, was normalized against β-galactosidase activity and expressed as RLU. Error bars indicate the SD of triplicate wells. Data are representative of three similar experiments.

FIGURE 6. In vivo HKBA induction of IL-12p40 is MyD88 dependent, but TLR2 and TLR4 independent. A, C57BL/6, TLR2−/−, MyD88−/− mice; B, C3H/HeOuJ and C3H/HeJ (TLR4 mutant) were injected with HKBA (10⁷/ml). Sera were collected at different time points and assayed for IL-12p40 production by ELISA. The values shown for PBS-injected mice are the means for the three different time points. *, p < 0.05, compared with WT and TLR2−/− mice. Data are representative of two similar experiments.
we have shown that *B. abortus* can induce mouse DC to migrate to T cell areas in the spleen, where they secrete IL-12 (30). Thus, the DC engage the adaptive arm of the immune system and set in motion a Th1-like response that influences the induction of CTL and Abs of a particular subclass profile (9, 10, 33).

The mechanism of innate immune recognition of *B. abortus* is unknown. Because several bacteria were previously shown to be recognized by the TLR family of receptors, we hypothesized that this family might be involved. To investigate this, we evaluated responses from animals that lack key components of the TLR activation cascade, such as the adapter molecule MyD88, or mice with mutated or with targeted deletion of certain TLRs, such as TLR2 and TLR4. We demonstrate that, indeed, *B. abortus*, like several other bacteria, activates cells, including DC, to secrete proinflammatory cytokines, such as IL-12 and TNF, under a TLR-dependent pathway. Interestingly, induction of TNF and IL-12p40 involves different TLRs in that TLR2 was required for TNF, but not IL-12p40 production. TLR4 was not required for either TNF or IL-12p40 induction by HKBA.

Experiments were also performed with hamster and human cells transfected with different TLRs and reporter genes that respond to NF-kB activation, namely CD25, luciferase. Results show that HKBA activates human TLR2/NF-kB in a dose-dependent manner, but does not activate TLR4 or TLR9. This is in agreement with previous data indicating that LPS and DNA from *B. abortus* are relatively inefficient in eliciting cytokine production (19, 21). Thus, an early event following HKBA activation of the innate immune system, namely TNF induction, occurs via TLR2 and NF-kB activation.

In other systems, TLR2 activation also required TLR6 and/or TLR1 (34–37). This may also be the case for HKBA, but transient transfection experiments with combinations of TLR2 with either TLR6 or TLR1 did not show significant enhancement (data not shown). The possible association of TLR6 and TLR1 in the TLR2 molecular complex for signaling triggered by HKBA stimulation is currently under investigation in our laboratory.

Murine splenocytes produced significant levels of IL-12p40 following HKBA stimulation in vitro. Enriched DC from MyD88−/− mice failed to secrete this cytokine; however, cells from TLR2−/− or TLR4−/− mice produced high levels of the cytokine when stimulated with this bacterium. This interesting observation led us to conclude that IL-12 induction is MyD88 dependent, but, unlike TNF responses, does not involve TLR2 or TLR4. Other known TLRs, namely 1, 6, and 9, do not appear to play a role because no signaling of these TLRs was detected following triggering of cells with HKBA transiently transfected with these TLRs (data not shown). Furthermore, IL-1 and IL-18 involvement was ruled out by treating HKBA-stimulated cells with neutralizing Abs. Taken together, these results depict how *B. abortus* interacts with the innate immune system by triggering several TLRs and eliciting different responses, depending on which receptor is triggered. Early activation of TNF requires triggering via TLR2 and involves NF-kB activation, whereas up-regulation of IL-12p40 involves the MyD88 pathway, but does not require TLR2, 4, or 9.

Future experiments will focus on identification of the molecules on the surface of HKBA that bind and activate TLRs. Although LPS from many bacteria tested act via TLR4 and are inhibitable by polymyxin B, there are LPS preparations from some bacteria that act via TLR2 (38–41). LPS from *B. abortus* is an unlikely candidate because it is a poor stimulator of TNF secretion and acts via TLR4. Moreover, TNF responses to HKBA were not inhibitable by polymyxin B (unpublished observation). Similarly, DNA from *B. abortus* is much less active than HKBA in its ability to induce TNF (unpublished observation). Furthermore, we show that TLR9, which has been previously shown to be the receptor for bacterial DNA (29, 42), is not required for HKBA induction of TNF. Lipoprotein from *B. abortus* may be involved in triggering via TLR2 because other microbial lipoproteins activated cells in a TLR2-dependent manner (43). Although soluble LPS from *B. abortus* acted on TLR4, intact HKBA did not stimulate TLR4. This may be due to the tight association between *B. abortus* LPS on the cell surface of HKBA with lipoproteins, which may stearically hinder interaction of the LPS with TLR4 (44, 45).

We have shown that the cell types that respond early to HKBA stimulation in terms of IL-12 secretion are DC (30). These cells express TLR2 and can be induced to secrete TNF by HKBA. Secretion of IL-12 has been shown to be important in development of the subsequent immune response both in terms of Th1 IFN-γ induction and Ab subclass profiles (46). Interestingly, the same splenic DC population used distinct pathways in responding to HKBA, such that the IL-12p40 response was dependent on MyD88, but not TLR2, whereas TNF secretion required both MyD88 and TLR2. Although HKBA mainly activates DC, macrophages can also be stimulated (30). Similar to DC, peritoneal macrophages respond to HKBA by induction of IL-12p40 in a TLR2-independent manner, whereas TNF induction is TLR2 dependent.

In vivo protection against *B. abortus* infection was shown to require both TNF and IL-12 (47). Taken together, the results presented in this work demonstrate a complex interaction between a pathogen and cells of the innate immune system, in which a single
receptor cannot be assigned as sole recognition molecule and trigger for all components of proinflammatory responses. To cope with the intricacy of sensing pathogens, the innate immune system probably evolved to respond via multiple interactions to maximize activation. Understanding these interactions may contribute to the design of adjuvants that optimally trigger innate immunity to improve the breadth of early and memory cell responses to pathogens.

Acknowledgments

We are grateful to Drs. Marina Zaitseva and Mike Kennedy for review of the manuscript, Dr. Seiichi Yamano for technical advice, and Sara Heiney for assistance.

References


