Toll-like Receptor (TLR) Signaling in Response to Aspergillus fumigatus*

Revised for publication, February 19, 2002, and in revised form, August 5, 2002
Published, JBC Papers in Press, August 8, 2002, DOI 10.1074/jbc.M201683200

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Aspergillus fumigatus causes life-threatening infections in patients with qualitative and quantitative defects in phagocytic function. Here, we examined the contribution of Toll-like receptor (TLR)-2, TLR4, the adapter protein MyD88, and CD14 to signaling in response to the three forms of A. fumigatus encountered during human disease: resting conidia (RC), swollen conidia (SC), and hyphae (H). Compared with elicited peritoneal macrophages obtained from wild-type and heterozygous mice, TLR2−/− and MyD88−/− macrophages produced significantly less tumor necrosis factor-α (TNFα) following A. fumigatus stimulation. In contrast, following stimulation with RC, SC, and H, TLR4−/− and CD14−/− macrophages exhibited no defects in tumor necrosis factor-α release. TLR2−/−, TLR4−/−, MyD88−/−, and CD14−/− macrophages bound similar numbers of RC and SC compared with wild-type macrophages. RC, SC, and H stimulated greater activation of a nuclear factor κ B (NFκB)-dependent reporter gene and greater release of tumor necrosis factor-α from the human monocytic THP-1 cell line stably transfected with CD14 compared with control cells stably transfected with empty vector. A. fumigatus stimulated NFκB-dependent reporter gene activity in the human embryonic kidney cell line, HEK293, only if the cells were transfected with TLR2. Moreover, activity increased when TLR2 and CD14 were co-transfected. Taken together, these data suggest that optimal signaling responses to A. fumigatus require TLR2 in both mouse and human cells. In contrast, a role for CD14 was found only in the human cells. MyD88 acts as a central adapter protein mediating signaling responses following stimulation with RC, SC, and H.

A. fumigatus is a saprophytic fungus ubiquitous in the environment (1). Human exposure most commonly occurs following inhalation of airborne resting conidia (RC),1 which are an ideal size for alveolar deposition. Bronchoalveolar macrophages can phagocytose and kill RC and thus are thought to constitute the first line of defense against the fungus. Should this initial defense fail, the RC become metabolically active and grow into swollen conidia (SC) and eventually hyphae (H), the invasive form of the fungus. Thus, the host can successfully defend against aspergillosis by killing any of the three growth phases of the fungus, RC, SC, and H. The spectrum of aspergillosis (2) ranges from allergic manifestations, mostly seen in atopic individuals, to invasive disease, which occurs almost exclusively in those with severe immunocompromise. Most patients with invasive aspergillosis have a qualitative or quantitative disorder of phagocyte function such as neutropenia due to chemotherapy or macrophage dysfunction due to high doses of corticosteroids (3). Even with antifungal therapy, invasive aspergillosis is associated with high mortality rates.

In Drosophila, the genes encoding antibacterial and antifungal peptides are differentially expressed following injection of distinct microorganisms. Drosophila that are naturally infected by A. fumigatus exhibit an adapted response by producing peptides, including drosomycin, with antifungal activity. This response is mediated through the selective activation of the Toll pathway. Drosophila with toll mutants are overwhelmed following challenge with A. fumigatus (4, 5). Mammalian cells contain toll-like receptors (TLR) with homology to Drosophila toll (6). At least ten members of the TLR family have been identified in humans and mice (7). The TLR is characterized by extracellular leucine-rich repeats and a cytoplasmic Toll/IL-1R domain that is shared with IL-1R family proteins, including the IL-1R, IL-18R, and T1/ST2 (8). The TIR homology domain is also found in the cytoplasmic adapter protein, MyD88, which interacts with the IL-1R/TLR family members. Stimulation via the IL-1R/TLR family leads to initiation of signaling cascades that culminate in activation of nuclear factor κ B (NFκB) and mitogen-activated protein kinases. This process facilitates the transcription of genes that regulate the adaptive immune response, including those for many cytokines and chemokines.

Recent studies have established that individual microbial ligands activate specific TLRs. Moreover, in many instances, activation requires the presence of a co-receptor, which may function as an initial binding receptor. This is best worked out for lipopolysaccharide (LPS) from Gram-negative bacteria. LPS

α: NFκB, nuclear factor κ B; TIR, Toll/IL-1R; WT, wild-type; PG, peptidoglycan; Bb, Borrelia burgdorferi; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; FBS, fetal bovine serum; IL, interleukin; ELISA, enzyme-linked immunosorbent assay; ELAM, endothelial-leukocyte adhesion molecule; HEK, human embryonic kidney cells; KO, knock out; RSV, respiratory syncytial virus.

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This paper is available on line at http://www.jbc.org
binds to the glycosylphosphatidylinositol-anchored cell protein, CD14, in the presence of a serum factor, LPS binding protein. However, signal transduction requires the presence of TLR4 (9–11). Other TLR4 ligands include the Cryptococcus neoformans capsular polysaccharide, glucuronoxylomannan (12). TLR2, on the other hand, has been shown to mediate cellular responses to microbial products derived from group B streptococcus (13), peptidoglycan (PG) and lipoteichoic acid from Gram-positive bacteria (14–16), lipoproteins/lipopeptides from Mycobacterium and Borellia burgdorferi (Bb) (17–19), and ymosan (20). In many of these cases, TLR2 forms a multimer with either TLR1 or TLR6 (21, 22), thus diversifying the pattern of recognition. Other recently described TLR microbial ligands include TLR3, TLR5, TLR7, and TLR9, which signal in response to double-stranded RNA, bacterial flagellin, small antiviral compounds, and unmethylated CpG DNA, respectively (23–25).

The inflammatory response is critical to the survival of all complex organisms, serving to eliminate or isolate the injurious agent(s) and facilitate the repair and regeneration of damaged tissue (26). The proinflammatory cytokine TNFα plays a critical role in the regulation of the inflammatory response following challenge by A. fumigatus. Thus, in murine models of aspergillosis, neutralization of TNFα is deleterious, whereas pre-treatment with a TNFα agonist peptide enhances resistance (27–29). TNFα appears to be particularly critical for neutrophil recruitment into infected organs. In a recent report, Wang et al. (30) demonstrated that human monocytes treated with blocking antibodies directed against either TLR4 or CD14 had a modest reduction in TNFα production following stimulation with A. fumigatus hyphae. In the present study, the contribution of CD14 and TLRs signaing pathways to the production of TNFα following stimulation by A. fumigatus was examined using knockout mice and transfected cell lines. The three fungal forms, RC, SC, and H, which the host is exposed to during the course of an infection, were used as stimuli. We found that TLR2 is the predominant cell surface receptor required for signaling in murine macrophages and HEK293 cells, whereas activation of NFκB was enhanced in THP-1 cells and HEK293 cells in the presence of CD14. Moreover, signaling occurs mainly via the adapter protein MyD88.

MATERIALS AND METHODS

Materials—All reagents were obtained from Sigma, unless stated otherwise. Phosphate buffered saline (PBS) and RPMI 1640 were purchased from BioWhittaker (Walkersville, MD). Heat-inactivated fetal bovine serum (FBS), L-glutamine, HEPES, LPS from Escherichia coli O111:B4 (smooth) was subjected to a modified phenol re-extraction protocol (35). This resulted in a product with TLR4, but not TLR2, agonist activity. Listeria monocytogenes (Lm) was used at a final microbe to cell ratio of 1:1. PG was used at a concentration of 10 μg/ml B. burgdorferi (Bb) lysate was used at a concentration of 1 μg/ml (36). Thioglycollate medium without indicator and Sabouraud dextrose agar were obtained from Remel (Lenexa, KS). Lm, PG, and Bb were prepared as described (14, 16, 36).

Peritonal Macrophages—MyD88−/− (37), TLR2−/− (38, 39), TLR2+/−, and CD14−/− (40) mice were engineered as described on a C57BL/6 background. Wild-type (WT) C57BL/6, C3H/HeOuJ (hereafter referred to as C3H/OUJ), and C3H/HeJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Peritoneal macrophages were isolated from 8–10-week old mice (36). Briefly, the mice were injected intraperitoneally with 3 ml of thioglycollate, and after 4 days peritoneal exudate cells were harvested by lavage with 10 ml of RPMI 1640 medium supplemented with 10% heat-inactivated FBS and 10 μg/ml ciprofloxacin. The cells were washed once in the medium and plated at a density of 1 × 10^5 per well in a 96-well tissue culture plate. After 2 days, non-adherent cells were washed free, fresh medium was added, and the adherent cells were challenged with the indicated stimuli for 18 h. Supernatants were then harvested and tested for TNFα release by ELISA as described above. The cells from the CD14−/− mice and their WT counterparts were harvested and cultured in serum-free medium (Invitrogen) so as to avoid the confounding effects of soluble CD14 present in serum (41). These cells did not encounter serum at any time during the course of the experiment.

Cells and Transfections—The human myeloid cell line, THP-1, stably transfected with glycosylphosphatidylinositol-anchored CD14 (THP1-CD14 expressing 2 × 10^6 molecules per cell) and control THP-1 cells stably transfected with the empty RSV vector (THP1-RSV) were kindly provided by Dr. Richard Ulevitch (Scirpps, La Jolla, CA) (42). THP1-RSV cells express minimal (<1000 molecules/cell) amounts of cell surface CD14 (42). TLR2 and TLR4 are constitutively expressed on THP1 cells, and the expression does not change when the cells are matured (43). The cell lines were maintained in complete medium supplemented with 10% FBS and 0.5 μg/ml geneticin. A luciferase reporter gene (44) from pGL3 under control of NFκB- and EGFP-dependent ELAM-1 promoter (pELAM.luc) was purified from E. coli J109 using EndoFree Plasmid kit (Qiagen). THP1-CD14 and THP1-RSV cells were transiently transfected with the ELAM-luciferase reporter plasmids using DEAE-Dextran and 1 μg of plasmid DNA per 1 × 10^6 cells, as in our previous study (45). Twenty-four hours following transfection, the cells were challenged for 18 h with the indicated stimulus, LPS, or other stimuli as measured in cell lysates using a kit from Promega and read on a luminometer.

HEK293 cells (ATCC, Manassas, VA) were maintained by serial passage in Dulbecco’s Modified Eagle’s Medium (BioWhittaker, Walkersville, MD) supplemented with 10% FBS, L-glutamine, and ciprofloxacin. By RT-PCR, HEK293 cells express mRNA for TLR1 and TLR6, but not for TLR2 and TLR4 (46). Cells were transiently transfected using Polyfect (Qiagen) according to the manufacturer’s protocol. In addition to pELAM.luc, plasmids used for transfection included ones containing genes encoding for TLR2, TLR4 (15), CD14 (47), and MD2 (48).

Statistics—Means and Standard Errors (S.E.) were derived using a Statistics—Means and Standard Errors (S.E.) were derived using a

A. T. Golenbock, unpublished data.
natants were tested for TNFα release by ELISA. All the stimuli showed a significant difference in TNFα release when comparing the WT to the MyD88−−/− cells (p < 0.0001). Data represent means ± S.E. of five separate experiments, each performed in triplicate.

statistical software program (SigmaStat; Jandel Scientific Software, San Rafael, CA). The two-tailed Student’s t test was used to compare experimental groups. The Bonferroni correction was used when multiple comparisons were made.

RESULTS

The Role of MyD88 in Signaling TNFα Production in Response to A. fumigatus—MyD88 is a cytoplasmic adapter protein that appears essential for optimal signaling via TLR2 and TLR4 (37). To determine the role of MyD88 in aspergillosis, TNFα release from peritoneal macrophages obtained from MyD88−−/− mice was determined. The three growth phases of A. fumigatus, as well as LPS and PG, stimulated significantly higher TNFα release from the WT macrophages compared with the MyD88−−/− macrophages (Fig. 1) (p < 0.0001). These data demonstrate that the vast majority of Aspergillus-stimulated TNFα release is dependent upon signaling pathways utilizing MyD88.

The Role of TLR2 in the TNFα Production in Response to A. fumigatus—Elicited peritoneal macrophages from TLR2−−/− mice and their TLR2+−/− littermates were examined for their ability to secrete TNFα in response to stimulation by RC, SC, and H of A. fumigatus. As positive controls for each of these experiments, wells containing known TLR2 (PG) and TLR4 (phenol re-extracted LPS) agonists were included. Unstimulated macrophages served as negative controls. In selected experiments, to rule out endotoxin contamination, 20 μg of polymixin B per ml were added to the wells prior to stimulation. This had no significant effect on cytokine release in response to the fungal stimuli (data not shown). RC and H stimulated significantly more TNFα production from the TLR2−−/− macrophages compared with the TLR2+−/− macrophages (Fig. 2). In contrast, SC stimulated similar amounts of TNFα from TLR2−−/− and KO macrophages. As expected, the TLR2 agonist, PG, stimulated TNFα release only from the wild-type macrophages, whereas the TLR4 agonist, LPS, stimulated similar amounts of cytokine release from heterozygote and TLR2−−/− macrophages.

The Role of TLR4 in the TNFα Production in Response to A. fumigatus—C3H/HeJ mice have a point mutation in the TIR domain of TLR4 rendering them hyporesponsive to LPS (50, 51). Secretion of TNFα in response to Aspergillus stimulation was compared in elicited peritoneal macrophages from C3H/HeJ mice and congenic TLR4−−/− C3H/OuJ mice (Fig. 3). There were no significant differences in TNFα release between the macrophages from the two strains following stimulation with RC, SC, and H. However, there was a trend, albeit not significant (p = 0.072), toward greater TNFα release from the C3H/OuJ macrophages following stimulation with SC. The TLR2 agonist, PG, stimulated similar amounts of TNFα from both cell types, whereas the TLR4 ligand, LPS, only stimulated the wild-type macrophages. These data suggest that TLR4 does not play a role in signaling murine macrophage TNFα release following stimulation by RC and H, although a small contribution of TLR4 in signaling responses to SC cannot be excluded.

The Role of CD14 in the TNFα Production in Response to A. fumigatus—To study the role of CD14 in signaling cytokine responses to A. fumigatus, peritoneal macrophages obtained from CD14−−/− mice were stimulated with the three growth phases of Aspergillus and TNFα release determined (Fig. 4). These experiments were performed using serum-free medium because serum contains soluble CD14 (52, 53). There were no significant differences seen in TNFα release upon stimulation by RC, SC, and H when comparing CD14−−/− macrophages to the WT. As expected, the positive controls LPS and Bb showed a significant increase in TNFα release in the WT compared with the CD14−−/− (p < 0.0001). These data suggest that signaling in Aspergillus is not dependent upon CD14 in mice.
The Role of Human CD14 in the Activation of NFkB and Release of TNFα in Response to *A. fumigatus* Stimulation—The above experiments suggested that CD14 was not required for mouse macrophage responses to *A. fumigatus*. These data contrast with those of Wang et al. (30), showing a role for CD14 in human monocyctic responses to hyphal stimulation. To investigate whether species-related differences could account for this disparity, the role of CD14 was examined further by studying TNFα release and NFkB nuclear translocation in the human myelomonocytic cell line, THP1, stably transfected with CD14 (THP1-CD14). THP1 cells containing an empty RSV vector (THP1-RSV) served as control cells. The cells were transiently transfected with a plasmid, pELAM.luc, containing an NFkB-dependent promoter driving expression of luciferase and then stimulated with *A. fumigatus* (Fig. 5A). The three growth phases of the *A. fumigatus*, RC, SC, and H, stimulated significantly higher NFkB production, as measured by luciferase production, in the THP1-CD14 cells than in the THP1-RSV cells ($p < 0.001$). Similarly, LPS, which binds to CD14 (10), also stimulated significantly more luciferase in the THP1-CD14 than in the THP1-RSV cells ($p < 0.001$). Data represents means ± S.E. of three separate experiments, each performed in triplicate.

Because NFkB activation does not necessarily lead to TNFα release (12), we assayed TNFα release from THP1-CD14 and THP1-RSV stimulated with *A. fumigatus* and, as a positive control, LPS (Fig. 5B). RC, SC, and H of *A. fumigatus*, as well as LPS, stimulated significantly higher TNFα production in the THP1-CD14 cells compared with the THP1-RSV cells ($p < 0.003$). In contrast, *L. monocytogenes* stimulated comparable amounts of TNFα from both cell lines. Differentiation of THP1-RSV cells for 3 days with 100 ng/ml LPS, or B. burgdorferi (Bb) for 18 h. The supernatants were tested for TNFα release by ELISA. Comparing the two populations of macrophages, there were significant differences in TNFα release only following stimulation with LPS and Bb ($p < 0.001$). Data represents means ± S.E. of three separate experiments, each performed in triplicate.

**Fig. 4.** TNFα release from peritoneal macrophages of CD14+/− and WT mice stimulated with *A. fumigatus*. Peritoneal macrophages were left unstimulated (UNS) or stimulated with RC, SC, H, 100 ng/ml LPS, or B. burgdorferi (Bb) for 18 h. The supernatants were tested for TNFα release by ELISA. Comparing the two populations of macrophages, there were significant differences in TNFα release only following stimulation with LPS and Bb ($p < 0.001$). Data represent means ± S.E. of three separate experiments, each performed in triplicate.

**Fig. 5.** NFkB nuclear translocation and TNFα release by THP1-CD14 and THP1-RSV cells stimulated with *A. fumigatus*. A, cells were transfected with the NFkB-dependent reporter plasmid, pELAM.luc, and then challenged for 18 h with the indicated stimuli. Concentrations of LPS and TNFα were 100 ng/ml and 5 ng/ml, respectively. Luciferase activity was measured as described under “Materials and Methods.” RC, SC, H, and LPS stimulated significantly greater luciferase in THP1-CD14 compared with THP1-RSV cells ($p < 0.001$). Data represents ± S.E. of three separate experiments, each performed in triplicate. B, cells were incubated with the indicated stimuli for 18 h, and then TNFα was measured by ELISA. RC, SC, H, and LPS stimulated significantly greater TNFα release from THP1-CD14 compared with THP1-RSV cells ($p < 0.001$). Data represent means ± S.E. of two separate experiments, each performed in triplicate.

**Human Embryonic Kidney Cells**—The above experiments suggested that human and murine cells might utilize different receptors to signal responses to *A. fumigatus*. To examine this issue further, HEK293 cells were transiently transfected with the NFkB-dependent reporter plasmid, pELAM.luc, along with plasmids containing the genes for either TLR2 or TLR4 alone or in combination with CD14 (Fig. 6). The transfected cells were then stimulated with *A. fumigatus*, and luciferase activity was measured. There was activation of the NFkB reporter by RC, SC, and H in HEK293 cells transfected with TLR2. Addition of CD14 resulted in an enhanced response compared with that seen with TLR2 transfection alone. However, transfection with TLR4, alone or with CD14, did not induce NFkB activation. HEK293 cells transfected with CD14 alone also did not respond to *Aspergillus* stimulation (data not shown). LPS, as previously demonstrated (48, 54), only activated NFkB in the presence of TLR4, CD14, and MD2. All the transfected HEK293 cells responded to the non-TLR ligand, IL-1β (data not shown).
these receptors were required for binding to RC and SC (Fig. 7). Mouse peritoneal macrophages mutant in TLR2, TLR4, MyD88, or CD14 exhibited no significant defects in conidial binding compared with WT macrophages. Similarly, binding indices were similar comparing THP1-CD14 and THP1-RSV cells. HEK293 also showed no significant differences in binding (data not shown).

**DISCUSSION**

A critical component of host defenses against microbes is the ability of the immune system to recognize and respond to foreign invaders. Recent studies have established the central role of TLRs, often acting with CD14, in innate immune recognition of a wide variety of microbial pathogens. In the studies reported herein, the contribution of CD14, TLR2, TLR4, and the adapter protein MyD88 to signaling responses to the opportunistic fungus *A. fumigatus* was assessed. This study employed macrophages from KO mice as well as a transfected human myelomonocytic cell line and human embryonic kidney cell lines. Our data demonstrate that TLR2, CD14, and MyD88 all contribute to signaling responses to *A. fumigatus*.

Recently, Wang *et al.* (30) reported that monoclonal antibody directed against CD14 and TLR4, but not TLR2, partially inhibited TNFα release from human monocytes stimulated by ethanol-fixed, serum-opsonized *A. fumigatus* hyphae. Our data confirm the role of CD14, at least in human cells, but in our studies TLR2, rather than TLR4, was the dominant receptor necessary for signaling TNFα responses to hyphae in both the human cell line HEK293 and mouse peritoneal macrophages. The reasons for the differences between the studies are speculative. Wang *et al.* (30) inferred a role for TLR4 based on blocking studies with an anti-TLR4 monoclonal antibody, HTA125. However, at concentrations that inhibited LPS release by 85%, HTA125 inhibited hyphal-stimulated TNFα release by only 35%. Thus, their results suggest other signaling receptors are involved.

Our data using the human cell lines THP-1 and HEK293 are in agreement with those of Wang *et al.* (30) regarding a role for CD14 in signaling TNFα production in response to *A. fumigatus* hyphae. Those investigators used a blocking antibody directed against CD14 and demonstrated a 70% reduction in TNFα release from monocytes. In our studies, we demonstrated that THP1 and HEK293 cells transfected with CD14 released...
significantly more TNFα following A. fumigatus stimulation compared with control cells transfected with empty vector. THP1-RSV cells express only small amounts of CD14 (42). In addition to its well recognized role as an LPS receptor, CD14 has been implicated as a pattern recognition receptor for a wide variety of microbial and non-microbial ligands (55). Other fungal-derived ligands recognized by CD14 include the C. neoformans capsular polysaccharide, glucuronoxylomannan, and the Blastomyces dermatitidis adhesion, WI-1 (12, 56). All TLRs have a cytoplasmic TIR domain, which is necessary for signal transduction (57). Ligation of TLRs leads to activation of the NFκB and mitogen-activated protein kinase signaling pathways through cytoplasmic adapter proteins (58). Macrophages lacking the adapter protein MyD88 make little to no proinflammatory cytokines when challenged with a broad range of stimuli including LPS (37), Staphylococcus aureus (38), and taxol (59). In our studies, macrophages from mice deficient in MyD88 made ~ 90% less TNFα following A. fumigatus stimulation than did wild-type cells. The observation that A. fumigatus did stimulate detectable, albeit low, levels of TNFα from the MyD88−/− macrophages suggests that these fungal stimuli can utilize pathways independent of TLRs or adapter proteins other than MyD88. In this latter regard, two such adapter proteins, TIR domain-containing adapter protein (TIRAP) (57, 58) and Toll-interacting protein (Tollip) (60), were recently described. Moreover, it has been demonstrated that the cellular machinery distal to MyD88 is intact in the MyD88−/− mouse, including the ability to activate NFκB and mitogen-activated protein kinase pathways (37).

The three growth phases of A. fumigatus used in this study, RC, SC, and H, are those that the host encounters during clinical disease. Our data demonstrate growth phase-dependent differences between TLR utilization in the murine cells, whereas in the human cells, all phases required TLR2. Thus, TLR2 was of paramount importance for murine macrophage TNFα production stimulated by RC and H but not SC. Our data also showed that in human (but not murine) cells, CD14 contributed to NFκB activation stimulated by all three growth phases of Aspergillus. Although the reasons for these species-specific disparities remain speculative, there is evidence, discussed below, that murine and human phagocytes utilize different receptors to recognize A. fumigatus (61, 62).

Although fungal overgrowth precluded the use of live SC and H, we were able to use live RC. During the course of the 18-h incubation with macrophages, microscopic observation revealed that some of the RC germinated into H. Thus, the TNFα released following stimulation with RC reflects the contribution not only from RC but also from SC and H and shed fungal products. This models the situation in vivo where RC are inhaled and, if host defenses fail, germinate into H. One limitation, however, to the application of our studies to clinical disease is the use of peritoneal macrophages rather than the more relevant bronchoalveolar macrophages. The limited supply of KO mice precluded use of the latter cell type.

Although our data demonstrate that CD14, MyD88, TLR2, and perhaps TLR4 contribute to signaling TNFα in response to A. fumigatus, none of these proteins appears to be required for binding of the fungus to macrophages. This was evidenced by the finding that similar binding indices were obtained when comparing wild-type macrophages with macrophages deficient in these proteins. Moreover, although overexpression of CD14 in THP1 cells resulted in greater A. fumigatus-stimulated NFκB nuclear translocation and TNFα release, it had no significant effect on the binding indices. The finding that the receptors critical for cytokine production were distinct from those mediating binding is consistent with data from Underhill et al. (20). These investigators demonstrated that transfection of a macrophage cell line with a plasmid containing a gene encoding for a dominant-negative TLR2 resulted in inhibition of cytokine production but not phagocytosis in response to zymosan particles. Studies by Kan and Bennett (61, 62) have shown that macrophage β-glucan and mannosefucosyl receptor activation is necessary for binding of A. fumigatus to human monocytes and mouse alveolar macrophages, respectively. These data, taken together with the results presented herein, suggest that β-glucan and/or mannosefucosyl receptors function to bind A. fumigatus, whereas CD14 and TLR2 signal for TNFα.

The clinical implications of our study remain speculative. As discussed above, in experimental animal models, TNFα is critical for optimal host defenses against aspergillosis (27). Moreover, it has been demonstrated that transfection of the THP1-CD14 cell line.

ACKNOWLEDGMENTS—We thank Dr. Shizuo Akira for the TLR2 and MyD88 KO mice and for reading the manuscript. Dr. Richard Ulevitch kindly provided the THP1-CD14 cell line.

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