p38 MAPK Autophosphorylation Drives Macrophage IL-12 Production during Intracellular Infection

Leesun Kim, Laura Del Rio, Barbara A. Butcher, Trine H. Mogensen, Søren R. Paludan, Richard A. Flavell and Eric Y. Denkers


http://www.jimmunol.org/content/174/7/4178

**References**

This article *cites 55 articles*, 42 of which you can access for free at: [http://www.jimmunol.org/content/174/7/4178.full#ref-list-1](http://www.jimmunol.org/content/174/7/4178.full#ref-list-1)

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: [http://jimmunol.org/subscription](http://jimmunol.org/subscription)

**Permissions**

Submit copyright permission requests at: [http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: [http://jimmunol.org/alerts](http://jimmunol.org/alerts)
p38 MAPK Autophosphorylation Drives Macrophage IL-12 Production during Intracellular Infection

Leesun Kim,* Laura Del Rio,* Barbara A. Butcher,* Trine H. Mogensen,† Søren R. Paludan,† Richard A. Flavell,‡ and Eric Y. Denkers2*

The intracellular protozoan *Toxoplasma gondii* triggers rapid MAPK activation in mouse macrophages (MΦ). We used synthetic inhibitors and dominant-negative MΦ mutants to demonstrate that *T. gondii* triggers IL-12 production in dependence upon p38 MAPK. Chemical inhibition of stress-activated protein kinase/JNK showed that this MAPK was also required for parasite-triggered IL-12 production. Examination of upstream MAPK kinases (MKK) 3, 4, and 6 that function as p38 MAPK activating kinases revealed that parasite infection activates only M KK3. Nevertheless, in MKK3−/− M Φ, p38 MAPK activation was near normal and IL-12 production was unaffected. Recently, MKK-independent p38α MAPK activation via autophosphorylation was described. Autophosphorylation depends upon p38α MAPK association with adaptor protein, TGF-β-activated protein kinase 1-binding protein-1. We observed TGF-β-activated protein kinase 1-binding protein-1-p38α MAPK association that closely paralleled p38 MAPK phosphorylation during *Toxoplasma infection* of MΦ. Furthermore, a synthetic p38 catalytic-site inhibitor blocked tachyzoite-induced p38α MAPK phosphorylation. These data are the first to demonstrate p38 MAPK autophosphorylation triggered by intracellular infection. *The Journal of Immunology*, 2005, 174: 4178–4184.
MKK3, 4, or 6 that are known to be the upstream p38-activating MKK. Instead, we show that T. gondii, in contrast to LPS, triggers a recently described pathway of activation involving TAB1-dependent autophosphorylation of p38a (27–29). Our results are the first to show the importance of p38 autoactivation during microbial infection.

Materials and Methods

Mice and parasites

Female C57BL/6 strain mice (6–8 wk of age) were obtained from Taconic Farms and JNK2−/− and TLR2−/− mice were purchased from The Jackson Laboratory. The targeting strategies for MKK3−/− mice have been described previously (18). Colonies of JNK2−/−, TLR2−/−, and MKK3−/− animals were established in the Cornell University College of Veterinary Medicine animal facility. RH strain tachyzoites of T. gondii were maintained in human foreskin fibroblast monolayers by biweekly passage in DMEM (Invitrogen Life Technologies) supplemented with 1% heat-inactivated FCS (HyClone), 100 U/ml penicillin (Invitrogen Life Technologies), and 0.1 mg/ml streptomycin (Invitrogen Life Technologies). Parasite cultures were tested every 4–6 wk for Mycoplasma contamination using a commercial PCR-ELISA-based kit (Roche Applied Science). Soluble tachyzoite Ag (STAg) was prepared from tachyzoites of T. gondii strain RH as previously described (30).

Reagents

The rabbit anti-phospho-specific p38, SAPK/JNK, ERK1/2, MAPKAPK2, MKK3/6, MKK4, and c-Jun Ab, and rabbit anti-p38 Ab were purchased from Cell Signaling Technology, mouse anti-p30 (SAG-1) Ab was obtained from Argene, and rabbit anti-p38 Ab as well as goat anti-TAB1 Ab were obtained from Santa Cruz Biotechnology. The inhibitors SB202190 and SB203580 were from Calbiochem, U0126 was from Cell Signaling Technology, and SP600125 was purchased from Biomol Research Laboratories. Ultra-pure LPS from Salmonella minnesota R595 was purchased from List Biological Laboratories. Anisomycin was obtained from Sigma-Aldrich and protein A-Sepharose was purchased from Amersham Biosciences.

Cell culture

Bone marrow-derived Mϕ, prepared as described previously (25), and murine Mϕ cell lines were cultured in DMEM supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), and 3% HEPES (1 M). For in vitro infection, RH strain tachyzoites or LPS were added to Mϕ in plates (Costar, Corning, NY) and plates were briefly centrifuged (3 min, 200 × g) to synchronize contact between Mϕ and parasites. Samples were subsequently collected for biochemical analyses or cytokine ELISA.

Immunofluorescence microscopy

Bone marrow-derived Mϕ were allowed to adhere on sterile 12-mm coverslips in 24-well plates for 3 h. After 30 min of T. gondii infection (1:3 ratio of cells to parasites), Mϕ were fixed with 3% paraformaldehyde for 10 min and permeabilized and blocked simultaneously with 5% normal goat serum (Jackson ImmunoResearch Laboratories) in PBS with 0.075% saponin (Sigma-Aldrich). After overnight incubation at 4°C with rabbit primary Ab, mouse anti-p30 Ab was added for an additional 40 min at room temperature. After washing, cells were incubated for 45 min with Alexa Fluor 594, labeled goat anti-rabbit, or Alexa Fluor 488-conjugated goat anti-mouse Ab ( Molecular Probes). Nuclei were stained with 4′,6′-diamidino-2-phenylindole (Kirkgaard and Perry Laboratories) for the last 10 min of incubation in secondary Ab. After washing, coverslips were mounted with ProLong Antifade reagent ( Molecular Probes). Images were collected with a BX51 fluorescence microscope (Olympus) equipped with a DP 70 camera using DP controller software (version 1.1.1.65; Olympus) and DP manager software (version 1.1.1.71; Olympus).

Stable transfectants

Dominant-negative mutants of p38 and TAK1 were generated by stable transfection of J774A.1 murine Mϕ as described previously (31).

Immunoprecipitation

To detect phosphorylated p38a, wild-type (WT) and MKK3−/− Mϕ were infected with T. gondii or stimulated with LPS, cells were washed with PBS, then 0.4 ml of denaturing cell lysis buffer (50 mM Tris-HCl (pH 7.5), 70 mM 2-ME, and 1% SDS) was added. Immediately after, cells were harvested, 1.6 ml of ice-cold cell lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 μg/ml leupeptin, and 1 mM PMSF) was added and samples were boiled for 10 min. The supernatants were incubated with rabbit anti-p38a Ab (Cell Signaling Technology) with gentle rocking for 18 h at 4°C. Lysates were subsequently incubated with protein A-Sepharose for an additional 3 h at 4°C. Then p38a immunoprecipitates were washed three times with lysis buffer and once with ice-cold PBS. Samples were resuspended in reducing SDS sample buffer, boiled (3 min), and then subjected to immunoblot analysis for phospho- and total p38.

To detect p38 association with TAB1, Mϕ were infected with T. gondii or stimulated with LPS, then cells were washed with ice-cold PBS and incubated 10 min in ice-cold lysis buffer consisting of 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 μg/ml leupeptin, and 1 mM PMSF. Detergent extracts were clarified by centrifugation at 18,000 × g for 10 min at 4°C. The resulting supernatants were incubated with immobilized mouse anti-phospho-p38 Ab (Cell Signaling Technology) with gentle rocking for 18 h at 4°C. Then phospho-p38 immunoprecipitates were washed three times with lysis buffer and once with ice-cold PBS. Samples were resuspended in reducing SDS sample buffer, boiled (3 min), then subjected to immunoblot analysis.

Western blotting

Immunoblot analysis was performed as previously described (25).

Cytokine ELISA

Production of IL-12(p40) was measured by cytokine ELISA as described previously (32).

Statistics

The statistical significance of the data was determined by unpaired Student’s t test. Values of p < 0.05 were considered significant. All experiments were performed three or more times.

Results

Toxoplasma-induced IL-12 requires activation of MAPK signaling components

We examined activation of MAPK signaling components during infection of bone marrow-derived Mϕ by immunofluorescence microscopy (Fig. 1). Infected cells displayed phosphorylated p38 (Fig. 1, A and G), SAPK/JNK (B and H) and ERK1/2 (C and I) that were distributed throughout the cytoplasm and nuclei. In contrast, minimal levels of these MAPK were evident in noninfected cells (Fig. 1, M–O). We also found translocation of the MAPK substrates c-Jun (D and J), ATF-2 (E and K), and MAPKAPK2 (F and L) in infected Mϕ, unlike in noninfected cells (P–R).

We used pharmacological inhibitors to examine MAPK activation requirements in parasite-induced IL-12 production. As shown in Fig. 2A, SB202190 and SP600125, inhibitors respectively of p38 and SAPK/JNK kinase activities, prevented T. gondii-triggered production of IL-12(p40). In contrast, blocking ERK1/2 phosphorylation with U0126 resulted in a slight increase in IL-12. The latter result is consistent with reports suggesting a role for ERK1/2 in negatively regulating signaling pathways leading to IL-12 synthesis (33, 34). Phospho-specific Western blots (Fig. 2B) demonstrated the effectiveness of each inhibitor in blocking the activities of p38, SAPK/JNK, and ERK1/2. We also confirmed the specificity of each inhibitor using phospho-specific immunoblotting (data not shown).

Recent evidence suggests that the p38 MAPK inhibitor SB202190 displays inhibitory effects on tachyzoite replication (35). To avoid this potential adverse effect, we used a dominant-negative p38 Mϕ cell line (dnp38) to assess the requirement for host p38 in parasite-induced IL-12 production. The parental J774A.1 line produced significantly less IL-12 than bone marrow-derived Mϕ following infection (Fig. 2, A vs C). Nevertheless, parasite-induced production of the cytokine was totally obliterated.
in dnp38 cells (Fig. 2C). A similar p38 requirement was also found during stimulation with LPS (Fig. 2D). We used Mφ from JNK2−/− mice to evaluate the role of this SAPK/JNK molecule in IL-12 production. Absence of functional JNK2 had no effect on IL-12 synthesis induced during Toxoplasma infection. In contrast, LPS-triggered IL-12 release displayed a partial dependence upon JNK2. Taken together, the results show that T. gondii triggers Mφ IL-12 production through p38 and SAPK/JNK signaling pathway. LPS- but not Toxoplasma-induced IL-12 is partially JNK2-dependent.

Although LPS stimulation and T. gondii infection both provide a stimulus for IL-12 synthesis, the cytokine is produced with distinct kinetics in each case. As little as 6 h was required to induce maximal IL-12 levels during LPS stimulation (Fig. 3A). In contrast, parasite-induced IL-12 required 40 h to reach maximal levels. We recently reported that a soluble preparation of tachyzoite antigens (Fig. 3B, L, and R) or left uninfected (A–C). Parasites were visualized with mAb to SAG-1 (tachyzoite surface Ag-1) and Alexa Fluor 488-conjugated secondary Ab (green). MAPK signaling components were stained with Alexa Fluor 594-labeled secondary Ab (red). Cell nuclei were stained with 4′,6′-diamidino-2-phenylindole (blue). The fields in G–L are shown by differential interference contrast imaging of areas in A–F. After infection, phosphorylated p38, SAPK/JNK, and ERK1/2 are distributed throughout the cytoplasm and nuclei (A–C). Activated c-Jun, ATF2, and MAPKAPK2 display strong nuclear localization after infection (D–F). Tachyzoites cross-react with the anti-ERK1/2 and anti-MAPKAPK2 Ab and therefore appear yellow (C and F). The scale bar (in R) denotes 5 μm.

**FIGURE 1.** Activation of MAPK signaling components during T. gondii infection. Bone marrow-derived Mφ were infected with RH strain tachyzoites (A–L) or left uninfected (M–R). For infected cells, 30 min after tachyzoite addition, cells were fixed and stained with Ab to Toxoplasma and phospho-p38 (A, G, and M), phospho-SAPK/JNK (B, H, and N), phospho-ERK1/2 (C, I, and O), phospho-c-Jun (D, J, and P), phospho-ATF2 (E, K, and Q), and phospho-MAPKAPK2 (F, L, and R). Tachyzoites cross-react with the anti-ERK1/2 and anti-MAPKAPK2 Ab and therefore appear yellow (C and F). The scale bar (in R) denotes 5 μm.

T. gondii induces p38 phosphorylation in the absence of activated MKK3, MKK4, and MKK6

The p38 MAPK is phosphorylated by upstream kinases MKK3 and 6, as well as MKK4 (15). We used KO mice to examine role of MKK3, a major activator of p38, in parasite-induced p38 phosphorylation and IL-12 production. Even in MKK3−/− Mφ, both tachyzoites and LPS induced rapid p38 phosphorylation that was only marginally less than that obtained using WT cells (Fig. 4A). Using an Ab reactive with phosphorylated forms of both MKK3 and MKK6, we found no evidence for MKK6 activation in response to T. gondii or LPS, and as expected, MKK3 activation only in WT Mφ (Fig. 4A). This contrasted with anisomycin treatment that induced strong phosphorylation of both MKK3 and 6 in WT cells, and MKK6 activation only in KO Mφ. Fig. 4A also shows that while both LPS and anisomycin stimulate MKK4 activation, Toxoplasma does not induce phosphorylation of this MKK.

T. gondii induced IL-12 levels in MKK3-deficient Mφ that were not significantly different from WT cells (Fig. 4B). In line with previously published results, LPS-driven IL-12 production was decreased by ∼50% in the absence of MKK3 (18). Taken together, LPS-induced p38 phosphorylation that is required for IL-12 production (25) is likely to involve the combined activities of MKK3 and MKK4. In contrast, while p38 activation is required for parasite-induced IL-12, p38 phosphorylation can proceed in the absence of activated MKK3, 4, or 6.

TAK1 is not required for Toxoplasma-induced p38 activation but is necessary for parasite-triggered IL-12 production

The TAK1 molecule is a M3K family member implicated in activation of the IκB kinase complex and MKK proteins (22, 36). We used a dominant-negative approach to determine the role of TAK1 in parasite vs endotoxin-mediated p38 activation and IL-12 production. Fig. 5A shows that Toxoplasma activates p38 normally in dnTAK1 Mφ. Nevertheless, parasite-induced activation of MKK3, detected using an anti-phospho-MKK3/6 Ab, was defective in the dominant-negative TAK1 line. As with bone marrow-derived Mφ (Fig. 4), tachyzoite infection failed to elicit MKK4 activation in parent or dnTAK1 J774A.1 cells. The effects of LPS stimulation were distinct. Here, p38 was activated in the dnTAK1 Mφ to a lesser extent and with delayed kinetics relative to parental J774A.1 cells (Fig. 5A). This activity was paralleled by delayed MKK3 phosphorylation during LPS stimulation of the dominant-negative TAK1 line. In contrast, there was an almost complete failure of MKK4 activation in dnTAK1 Mφ.

Despite the fact that tachyzoites induced p38 activation in dnTAK1 Mφ, IL-12 production was reduced to background levels (Fig. 5B). This is presumably an indication that other TAK1-dependent kinases are required for parasite-induced IL-12 production. The production of IL-12 triggered by LPS was defective in the dnTAK1 cells (Fig. 5C). Together, the results shown in Figs. 4
and 5 argue that parasite-triggered p38 activation can proceed without activation of MKK3, 4, or 6.

Toxoplasma, but not LPS, induces p38 autophosphorylation

Recently, an alternative p38 activation mechanism that bypasses MKK-dependent MAPK phosphorylation was described. This involves autophosphorylation of the p38α isomer dependent upon association with TAB1 (28). We sought to determine whether Toxoplasma used this p38 activation pathway, making use of the pharmacological inhibitor SB203580. This molecule blocks p38α kinase activity, but does not prevent p38α transphosphorylation mediated by upstream MKK (37). Accordingly, we infected or LPS-stimulated WT and MKK3 KO Mφ in the presence and absence of SB203580, then immunoprecipitated p38α MAPK and immunoblotted with phospho-specific p38 Ab. Fig. 6A shows that catalytic-site inhibition of p38 prevents tachyzoite-induced p38 MAPK activation in both MKK3+/− and MKK3−/− cells, yet has minimal effect on LPS-induced p38 phosphorylation. Control blots using Ab against total p38 MAPK confirmed presence of the α isoform in immunoprecipitates from infected Mφ (Fig. 6A).

We then asked whether Toxoplasma infection triggered interaction between p38 and TAB1, an association predicted for p38 activation through autophosphorylation. Accordingly, Mφ were infected with T. gondii or stimulated by LPS, then p38 was immunoprecipitated using phospho-specific Ab. Immunoprecipitates were subsequently analyzed by Western blotting with anti-TAB1 and anti-p38 Ab. Fig. 6C demonstrates that TAB1 coprecipitates with p38 in lysates from tachyzoite-infected cells. In contrast, during LPS stimulation there was no evidence for TAB1 association with p38, despite the fact that the total amount of active p38 during LPS stimulation exceeded that occurring during Toxoplasma infection (Fig. 6C). Together, the results support a model in which T. gondii induces Mφ IL-12 production using a mechanism involving TAB1-dependent p38 autoactivation, bypassing conventional MKK-driven activation of this MAPK.

Discussion

The MAPK signaling cascades are important in both innate and acquired host defense mechanisms (15). Here, we show that p38 is
essential for IL-12 induction by the intracellular microbial pathogen *T. gondii*. Recently, soluble *Toxoplasma* extracts were also shown to induce Mφ IL-12 in dependence upon p38 MAPK, although in that case involvement of upstream MKK was not examined (38). Live parasite-induced p38 activation most likely involves a recently described mechanism involving TAB1-mediated autophosphorylation rather than MKK-mediated transphosphorylation (28, 29). As such, our data are the first to provide evidence that p38 autophosphorylation is an important activation pathway during microbial infection.

Activation of MAPK involves dual phosphorylation of threonine and tyrosine residues on a conserved regulatory TXY loop. Upstream MKK3 and MKK6 are important in p38 activation (36, 39–45). In addition, MKK4, a member of the SAPK/JNK signaling module, activates p38 under some conditions (46, 47). Here,

**FIGURE 5.** Role of TAK1 in p38 activation and IL-12 production. *A*, Activation of p38 in the absence of functional TAK1. J774A.1 Mφ and dominant-negative TAK1 cells derived from J774A.1 (dnTAK1) were infected with *T. gondii* (Tg; 6:1 ratio of parasites to cells) or stimulated with LPS (100 ng/ml) and anisomycin (25 μg/ml), then at the indicated time points, lysates were prepared for Western blot analysis using Ab specific for phospho-p38, phospho-MKK3/6, phospho-MKK4, and total p38 as a protein loading control. *B*, Requirement for TAK1 in *Toxoplasma*-driven IL-12 production. Parent and dnTAK1 cells were infected with *Toxoplasma* (Tg; 3:1 ratio of parasites to cells), and 36 h later supernatants were harvested for IL-12 ELISA. Med, cells cultured in medium alone. *C*, As in *B*, using LPS (100 ng/ml).

**FIGURE 6.** Evidence for p38 autophosphorylation during *Toxoplasma* infection. *A*, Catalytic-site inhibitor SB203580 blocks parasite-induced p38α phosphorylation. Bone marrow-derived WT and MKK3 KO Mφ were preincubated 30 min in the presence of SB203580 (2 μM) or in solvent alone (DMSO), then infected (Tg; 6:1 ratio of tachyzoites to cells) or LPS stimulated (100 ng/ml). At 10 and 20 min postinfection and 10 min post-stimulation, cell lysates were collected and subjected to immunoprecipitation with anti-p38Ab. The precipitates were subsequently probed by immunoblotting with Ab to phospho-p38 and reprobed with Ab to total p38. *B*, Association of TAB1 with p38 in parasite infected, but not LPS-stimulated cells. Bone marrow-derived Mφ were infected with *Toxoplasma* (Tg; 6:1 ratio of parasites to cells) or LPS stimulated (100 ng/ml). Samples were collected at the indicated time points and subjected to p38 immunoprecipitation using phospho-specific Ab. Precipitates were examined by Western blot analysis using anti-TAB1 and anti-p38Ab.
LPS stimulation led to partially reduced p38 activation and decreased IL-12 production in MKK3−/− bone marrow-derived Møs, results that are in line with previously published findings (18). In contrast to anisomycin stimulation, neither LPS triggering nor Toxoplasma infection induced significant MKK6 activation in WT or KO cells. For LPS stimulation, p38 phosphorylation may therefore occur through the combined activities of MKK3 and MKK4. The inability of T. gondii to activate MKK4 to detectable levels argues against this particular MKK in parasite-induced p38 activation.

Recently, MKK-independent p38 phosphorylation was described (28, 29, 48). This involves the scaffold protein TAB1 that normally functions in TAK1 activation (22, 49). The TAB1 molecule can associate with the p38α isomer, inducing autophosphorylation and activation of the latter. Although the molecular mechanism involved is not yet established, TAK1 and p38α binding involves distinct TAB1 domains, as shown by the recent identification of a TAB1 splice variant that binds and activates p38 in a TAB1 molecule:contrasting to anisomycin stimulation, neither LPS triggering nor Toxoplasma infection is the following. In WT cells, MKK3 was the sole p38 specific MKK activated during infection. However, MKK3 activation kinetics failed to match the p38 phosphorylation pattern, inasmuch as peak MKK3 phosphorylation levels were not attained until 60 min postinfection, a time by which p38 had undergone phosphorylation followed by dephosphorylation (Fig. 4) (25). In contrast, the kinetics of LPS-induced p38 phosphorylation closely paralleled both MKK3 and MKK4 activation. Furthermore, in Toxoplasma-infected MKK3−/− Møs, there was no detectable activation of MKK3, 4, or 6, yet p38 underwent normal levels of phosphorylation.

The pharmacological inhibitor SB203580 functions by occupying the ATP-binding pocket within the p38 kinase cleft, therefore acting to prevent downstream kinase activity rather than MKK-driven transphosphorylation (50). Thus, the finding that SB203580 blocks p38α phosphorylation triggered by T. gondii is evidence for p38 autophosphorylation. Furthermore, pull-down assays revealed a TAB1-p38 association that closely paralleled phosphorylation of the latter (Fig. 6). During LPS stimulation, we did not detect an effect of SB203580 on p38 activation, nor did we find evidence of TAB1-p38 association. Our results argue that LPS is likely to trigger MKK-driven p38 phosphorylation in bone marrow-derived Møs, but that TAB1-dependent autophosphorylation is a p38 activation mechanism triggered by T. gondii infection. The finding that Toxoplasma-induced IL-12 depends upon MyD88 argues that the latter upstream adaptor molecule is necessary in this process (6).

TAK1 is one of a large and diverse family of enzymes with M3K activity. Its property of activating both MAPK and IκB kinase cascades assigns it a potentially critical role in innate immune signaling pathways. The ability of T. gondii to induce normal p38 activity in Møs expressing a dominant-negative form of TAK1 provides additional evidence for MKK-independent MAPK activation (Fig. 5). In contrast, dominant-negative TAK1 cells displayed delayed kinetics of p38 and MKK3/6 phosphorylation during LPS stimulation. Insofar as MyD88-independent LPS signaling also involves delayed activation of MAPK pathways (51, 52), our results support the view that TAK1 functions in downstream signaling through MyD88.

Previous results have indicated Toxoplasma-induced inhibition of LPS-stimulated IL-12 and TNF-α that may relate to defects in endotoxin-triggered NFKb activation and MAPK activation (25, 32, 53). Nevertheless, as shown here, Møs eventually produce IL-12 during T. gondii infection. Therefore, it seems likely that Toxoplasma prevents proinflammatory cascades triggered by endotoxin, while simultaneously eliciting IL-12 through its own unique, partially overlapping transduction pathways. The finding that TNF-α remains potently suppressed at all time points during Mø infection suggests that this cytokine, and not IL-12, may be the true target of inhibition during Toxoplasma infection (54).

Production of IL-12 in T. gondii infected Møs occurred at lower levels and with distinct kinetics relative to LPS. Maximal amounts of IL-12 were achieved within 6 h of endotoxin stimulation. In contrast, parasite-induced IL-12 was not detected before 18 h and did not reach peak levels until 40 h postinfection. The delay in tachyzoite-elicited IL-12 could reflect a requirement for de novo synthesis of transcription factors driving IL-12 synthesis. In this regard, p38 is involved in CREB phosphorylation, the latter in turn increasing C/EBPβ transcription that is proposed as a factor for IL-12(p40) gene induction (13, 14). Increased C/EBPβ transcription only occurs after 2–4 h of LPS stimulation, and therefore other factors are likely involved in early endotoxin-induced IL-12 production (55). For Toxoplasma, a possible scenario is that the parasite is restricted to triggering IL-12 through a p38-mediated pathway involving CREB-dependent C/EBPβ synthesis. We are currently examining the role of these factors during T. gondii infection.

Why the parasite would display properties of triggering transduction cascades leading to induction of certain proinflammatory cytokines such as IL-12, while at the same time permanently blocking pathways leading to others such as TNF-α, is currently a matter for speculation. Regardless, the present study reveals that T. gondii-induced IL-12 requires p38 activation that is dependent upon parasite-triggered TAB1-dependent p38 autophosphorylation. As such, the data provide a physiological context for this unique mode of p38 activation that also suggests a potential target for therapeutic intervention.

Acknowledgments
We thank D. Holowka for insightful discussion and critical review of the manuscript.

Disclosures
The authors have no financial conflict of interest.

References


