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p38α Senses Environmental Stress To Control Innate Immune Responses via Mechanistic Target of Rapamycin

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The MAPK p38α senses environmental stressors and orchestrates inflammatory and immunomodulatory reactions. However, the molecular mechanism how p38α controls immunomodulatory responses in myeloid cells remains elusive. We found that in monocytes and macrophages, p38α activated the mechanistic target of rapamycin (mTOR) pathway in vitro and in vivo. p38α signaling in myeloid immune cells promoted IL-10 but inhibited IL-12 expression via mTOR and blocked the differentiation of proinflammatory CD4+ Th1 cells. Cellular stress induced p38α-mediated mTOR activation that was independent of PI3K but dependent on the MAPK-activated protein kinase 2 and on the inhibition of tuberous sclerosis 1 and 2, a negative regulatory complex of mTOR signaling. Remarkably, p38α and PI3K concurrently modulated mTOR to balance IL-12 and IL-10 expression. Our data link p38α to mTOR signaling in myeloid immune cells that is decisive for tuning the immune response in dependence on the environmental milieu. 

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Recognition of pathogen-associated molecular patterns by innate immune receptors triggers inflammatory and immune responses involving several signaling molecules including the MAPKs (1, 2). The MAPK p38α (also known as MAPK14) is one of four homologs of mammalian p38 and is essential in innate and adaptive immune signaling cascades (3). p38α is activated by diverse stimuli including TLR ligands, cytokines, and physicochemical stress signals such as UV irradiation, heat or osmotic shock, arsenite, or anisomycin (4). p38α is ubiquitously expressed in most cell types and regulates diverse functions such as cell proliferation, differentiation, apoptosis, tissue repair, tumorigenesis, or inflammation (4, 5). For example, p38α was previously identified as activator of the proinflammatory cytokines IL-1β, IL-6, TNF-α, or cyclooxygenase 2 (6–8). Several kinases are activated by p38α such as mitogen- and stress-activated kinases 1 and 2, MAPK-interacting serine/threonine-protein kinases 1 and 2, and MAPK-activated protein kinase (MK)2 and MK3 that mediates TNF-α production (9, 10).

The finding that inhibiting p38 blocks LPS-induced proinflammatory cytokine production (7) initiated the development of a wide range of p38 inhibitors for treatment of chronic inflammatory diseases such as rheumatoid arthritis, psoriasis, or Crohn’s disease (11). SB203580 is a competitive inhibitor of p38α and p38β by blocking ATP binding to the kinase, whereas BIRB796 is an allosteric inhibitor of p38α, p38β, and p38y (12, 13). Remarkably, so far, p38 inhibitors failed in clinical trials because of adverse and inflammatory effects such as liver toxicity or skin rashes (11). Recently, a more complex role of p38α has been reported (14–16). Expression of p38α in myeloid cells limits inflammation in an UV-induced irradiation model (14). These immunomodulatory effects of p38α may be mediated by the induction of the anti-inflammatory cytokine IL-10 (14, 15) and the inhibition of pro-inflammatory IL-12 (14, 16). However, the downstream pathway that controls coordinated IL-10 and IL-12 expression by p38α has remained elusive.

The classical insulin signaling pathway consisting of PI3K, Akt, and mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) has recently emerged as key regulator of innate immune cell homeostasis (17–20). Stimulation of innate immune cells by TLR ligands activates the mTOR pathway, and it is, in fact, a major pathway activated in LPS-stimulated macrophages based on phosphoproteomics (21). The function of PI3K–Akt–mTOR is cell type specific, but it has been shown that inhibition of PI3K by wortmannin or mTOR by rapamycin in myeloid cells, such as human monocytes, macrophages, or myeloid dendritic cells (DCs), enhances IL-12 production but blocks the release of IL-10 in vitro and in vivo (22–28). Tuberous sclerosis (TSC)2 is a tumor suppressor that is phosphorylated and inactivated by the protein kinase Akt, which itself is activated by...
PI3K (29). TSC2 forms a heterodimeric complex with TSC1 and negatively regulates mTOR (29). Conversely, knockdown of TSC2 in human monocytes or macrophages enhances IL-10 but inhibits IL-12 production (24, 30). In line, genetic inactiva-
tion of mTORC1 reduces IL-10 production in intestinal CD11c+ CD11b+ DCs (31). In contrast, TSC1-deficient macrophages show
elevated production of TNF-α and IL-12p40 (32). Despite these
observations, the precise regulatory units and upstream pathways
controlling mTOR-dependent cytokine production are still un-
clear.

An outstanding question is how myeloid immune cells adapt
and coordinate their immune response to an infectious trigger
toward the status of the environmental milieu, for example, how
to avoid detrimental tissue-destructive CD4+ Th1 responses
under conditions of tissue repair. Moreover, it is imperative to
explore the molecular signaling pathways that regulate p38α-
mediated immune responses for a deeper understanding of the
effects of p38 inhibitors for human health and disease. There-
fore, we tested whether p38α is connected to PI3K–TSC2–mTOR
signaling to regulate innate inflammatory responses. We found
that TLR ligands or environmental stress activates the TSC2-
mTOR pathway via p38α and MK2 to regulate the balance of IL-
12 and IL-10. Importantly, p38α acts in parallel to PI3K to control
the IL-12/IL-10 equilibrium in response to the environmental
milieu.

**FIGURE 1.** p38α modulates IL-12 and IL-10 in mice and humans. (A) p38αfl/fl and p38α−/− BMDM were stimulated with medium (−), LPS, SAC, or L.m. for 24 h. IL-12p40, IL-12p70, and IL-10 in the supernatants were determined by Luminex. Data are shown as means ± SEM for five mice. (B) Serum levels of IL-12p40, IL-12p70, and IL-10 were determined in p38α−/− and p38α−/− mice injected with LPS for 4 h (means ± SEM for three mice). (C-F) Human monocytes were treated with medium (−), BIRB796 (BIRB; 100 or 200 nM), SB203580 (SB; 200 nM or 2 μM), rapamycin (Rap; 100 nM) and then stimulated with (C) LPS, (D) SAC, (E) L.m., or (F) LPS and IFN-γ for 22 h. Cyto-
kines in cell-free supernatants were mea-
sured by Luminex (means ± SEM of at
least three donors). (G and H) Human monocytes were treated as indicated, washed, and added to allogeneic T cells for 1 wk. (G) IFN-γ of cell-free superna-
tants was determined by Luminex. Data represent means ± SEM for three inde-
pendent experiments. (H) Primed T cells were activated for 5 h with PMA/ion-
omycin. Intracellular cytokine staining for IL-4 and IFN-γ in CD-4 T cells is illustrated. One representative experiment out of three is shown. *p < 0.05 compared with the respective controls.

### Materials and Methods

**Reagents**

LPS (Escherichia coli O111:B4),wortmannin, anisomycin, and rapamycin were from Sigma. Staphylococcus aureus (SAC; PANSORBIN) and SD169 were from Calbiochem. BIRB796 was a kind gift of Sir Philip Cohen or purchased from Axon Medchem. SB203580 was from Tocris Bioscience and IFN-γ from R&D Systems. Heat-killed cells of Listeria monocytogenes (L.m.) were prepared by incubating the viable log-phase bacterial suspension at 70°C for 1 h. For UV exposure, cell culture plates were placed on a 20 × 20 UV-transilluminator (MWG Biotech) and activated with UV light for 10 s, 30 s, or 1 min.

**Cell culture**

Human PBMCs and peripheral human myeloid DCs were isolated as described previously (24). Monocytes were isolated from PBMCs by MACS using CD14 Microbeads (Miltenyi Biotec). RPMI 1640 supplemented with 2 mM l-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin (all from Life Technologies), and 10% FCS (HyClone) was used as culture medium. Mouse embryonic fibroblasts (MEFs) were cultured in DMEM containing 4.5 g/L glucose, 2 mM l-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, and 10% FCS. Tsc2−/−, p53−/−, and Tsc2−/− p53−/− as well as Tsc2−/−, Tsc1−/−, and Tsc1−/− MEFs were described previously (33, 34). p85α−/−, p85β−/− MEFs were a kind gift of Lewis Cantley. p38αfl/fl and p38α−/− mice were described previously (14). Bone marrow–derived macrophages (BMDMs) from mice were isolated and grown as described previously (35) and were replated 1 d before stimulation in full medium containing 2% FCS. MK2−/− immortalized murine macrophages stably reconstituted with MK2 or MK2βm were described previously (36).
Measurement of cytokine production

Cells were pretreated for 90 min with the indicated concentrations of SB203580, BIRB796, rapamycin, or wortmannin and then stimulated with 100 ng/ml LPS (+30 ng/ml IFN-\(\gamma\) as indicated), 75\(\mu\)g/ml SAC, or 10\(^7\) L.m. in 48-well plates. Cell-free supernatants were collected after 22–24 h as indicated. Human and murine cytokines were determined by the Luminex bead system with beads from R&D Systems and Affymetrix, and read on a Luminex 100 reader.

LPS injection

p38\(^{\Delta M}\) and p38\(^{\Delta 0.8}\) mice were housed and maintained at the Massachusetts General Hospital and Harvard Medical School. Mice were injected i.p. with 30\(\mu\)g/mouse LPS. After 4 h, serum samples were taken and spleens were isolated. Cytokine levels in the sera were measured by Luminex. Homogenization of mouse tissue was performed by using the Precellys-ceramic kit 2.8 mm and the Precellys 24 tissue homogenizer (both from peQLab).

T cell differentiation

Monocytes were incubated with medium, 200 nM BIRB796, 2 \(\mu\)M SB203580, or 100 nM rapamycin for 90 min and stimulated with 100 ng/ml LPS for 24 h. The cells were then washed with PBS and incubated with allogeneic T lymphocytes at a ratio of 1:1 in 24-well plates in RPMI complete medium. After 1 wk, IFN-\(\gamma\) production was determined in cell-free supernatants by Luminex. The primed cells were further activated for 5 h with 50 ng/ml PMA and 200 ng/ml ionomycin (both from Sigma) in the presence of 10\(\mu\)g/ml brefeldin A (Sigma) for the last 3 h. Afterward, cells were stained with FITC-labeled anti–IFN-\(\gamma\), PE-labeled anti–IL-4, and allophycocyanin-labeled anti-CD4 (all BD Bioscience) and analyzed by flow cytometry.

Analysis of signal transduction events

Monocytes, BMDMs, or 70% confluent MEFs starved overnight were treated and stimulated as indicated. Extract preparation and Western blotting were done as described previously (24). Abs were p-p70S6K (Thr389), p70S6K, p-4E-BP1 (Thr37/46), p-p38 (Thr180/Tyr182), p-S6 (Ser 240/244), p-Akt (Ser473), p-MK2 (Thr334), GAPDH, S6-ribosomal protein, p38 MAPK, p38\(\alpha\) MAPK, p38\(\beta\) MAPK, p38\(\delta\) MAPK, Tuberin/ TSC2 (all Cell Signaling Technology), p-Erk (Tyr204), I\(\kappa\)B\(\alpha\), and p38 (Santa Cruz Biotechnology).

Quantitative RT-PCR

RNA from human monocytes or murine immortalized macrophages was extracted in TRIzol (Invitrogen). cDNA was generated by Superscript II (Invitrogen). mRNA levels were determined by TaqMan Gene Expression Assays (Applied Biosystems) on a StepOnePlus Real-Time PCR System and normalized to ubiquitin.

Transfection of MEFs

Tsc2\(^{-/-}\) MEFs in six-well plates at 20–40% confluency were transfected in DMEM without antibiotics with 1 \(\mu\)g pcDNA3-HA-TSC2 wild-type (WT), pcDNA3-HA-TSC2 S1210A, or empty vector with Lipofectamine 2000 (Invitrogen) without antibiotics and FCS before stimulation.

Immunofluorescence microscopy

Cells were applied to eight-well Permanox chamber slides (Lab-Tek Chamber Slide System), fixed with 4% paraformaldehyde, quenched with 100 mM glycine, permeabilized with methanol, blocked with 1% BSA, and stained with FITC-labeled anti–p-S6, anti–p-MK2, or isotype control overnight at 4˚C. Cells were stained with Alexa Fluor 488–labeled goat anti-rabbit IgG (Invitrogen) followed by nuclear tracking using 0.1 \(\mu\)g/ml Hoechst-33342 (Invitrogen) and mounted in Vectashield mounting medium.

Statistics

Results are expressed as means \(\pm\) SEM. Student \(t\) test was used to detect statistical significance.
Results

p38α modulates IL-12 and IL-10 production in mice and human

To evaluate the role of p38α in the myeloid immune system, we generated BMDMs from mice with a deletion of p38α in cells expressing the lysozyme M gene (p38α<sup>fl/fl</sup>) and stimulated these cells with LPS, SAC, or heat-killed L.m. (Fig. 1A). Deficiency of p38α enhanced IL-12p40 and IL-12p70 expression, whereas the anti-inflammatory cytokine IL-10 was blocked compared with controls carrying homozygously the floxed p38α gene (p38α<sup>α/α</sup>; Fig. 1A). Other cytokines such as IL-1β, IL-23, or TNF-α were not significantly altered (data not shown). Injection of LPS into p38α<sup>Δ/Δ</sup> mice similarly deviated the production of IL-12 and IL-10 in vivo (Fig. 1B). Next, we characterized the precise function of p38α, which is the most highly expressed p38 isoform in human monocytes. We found that inhibition of p38 with different concentrations of SB203580 or BIRB796, as well as with the mTOR inhibitor rapamycin, strongly increased the production of IL-12p40 and IL-12p70 but blocked secretion of IL-10 after stimulation with LPS, SAC, or L.m. (Fig. 1C–F). Notably, SB203580 did not augment IL-12p40 production in L.m.-stimulated monocytes. Enhanced IL-12p40 but reduced IL-10 expression was also observed in SB203580- or BIRB796-treated peripheral human myeloid DCs stimulated with LPS, SAC, or L.m. (data not shown).

The surface expression of the costimulatory molecule CD86, important for T cell activation and priming, was enhanced upon p38 blockade in human monocytes after stimulation with LPS, SAC, or L.m. (data not shown). In line, we found that inhibition of p38 or mTOR in monocytes stimulated with LPS strongly enhanced the production of IFN-γ (Fig. 1G) and the differentiation of CD4<sup>+</sup> Th1 cells (Fig. 1H) in an allogeneic T cell activation model. In summary, these data show that p38α differentially regulates the expression of IL-12 and IL-10 in human monocytes and DCs, as well as in BMDMs in vitro and in vivo in response to microbial insult.

p38 activation stimulates mTOR signaling

The concurrent regulation of IL-12/IL-10 by p38 and mTOR inhibitors indicated that these molecules might be connected. Therefore, we explored whether inhibition of p38 or mTOR might mutually influence the other kinase. Rapamycin did not modulate the phosphorylation of p38 or its downstream kinase MK2 after stimulation of human monocytes with LPS but blocked the phosphorylation of the mTOR substrates p70S6K and 4EBP1 (Fig. 2A). In contrast, inhibition of p38 with either SB203580 or BIRB796 blocked MK2 activation as expected, but also decreased the phosphorylation of 4EBP1 and p70S6K, as well as S6, suggesting that p38 activates mTOR signaling. Total levels of the investigated proteins were not altered by treatment with the inhibitors (data not shown). Interestingly, phosphorylation of Akt at Ser473 was blocked, whereas activation of Erk was enhanced with BIRB796 and SB203580 (Fig. 2A, 2C). However, inhibition of Erk did not inhibit mTOR and did not modulate the production of IL-12 or IL-10 (data not shown). Degradation of IκB-α was not influenced by either p38 or mTOR (Fig. 2A). Activation of p38 by the two environmental stress signals anisomycin or UV also activated mTOR signaling in human monocytes in a p38-dependent manner (Fig. 2B, 2C). SD169, another reported inhibitor of p38α, was without effect in monocytes (Fig. 2A, 2C). Next, we tested whether hyperactivation of p38 with anisomycin in the presence of LPS or SAC could influence cytokine expression. Indeed, anisomycin reduced IL-12p40 mRNA levels but increased IL-10 mRNA levels in LPS- or SAC-stimulated macrophages (Fig. 2D, 2E). Remarkably, hyperactivation of p38 with anisomycin further increased mTOR activity in LPS-activated monocytes (Fig. 2F). These results indicate that p38 activates the mTOR pathway and thereby regulates the expression of IL-12 and IL-10.

p38α deletion blocks mTOR activation in vitro and in vivo via MK2

To extend our results genetically, we examined the mTOR signaling pathway in p38α<sup>Δ/Δ</sup> BMDMs. Deletion of p38α in macrophages abolished MK2 activation and also strongly diminished the phosphorylation of p70S6K, S6, and 4EBP1 after stimulation with...
LPS, UV, anisomycin, or L.m. (Fig. 3A and data not shown). Erk activation was observed only after LPS or L.m. treatment and not modified in p38α−/− MDMs (Fig. 3A). Moreover, activation of p70S6K and 4EBP1 was blocked in spleens from p38α−/−, but not p38α−/−, mice that were challenged with LPS (Fig. 3B), demonstrating that p38α activates mTOR signaling in vitro and in vivo. Next we analyzed whether MK2 may mediate the effect of p38α on mTOR signaling. Therefore, we used macrophages expressing a catalytic-dead mutant of MK2 (K79R) or its WT control. Strikingly, activation of mTOR after stimulation with LPS or anisomycin was severely compromised in the K79R mutant compared with WT MK2 (Fig. 3C). Moreover, the K79R macrophages showed absent IL-10 expression after LPS or SAC stimulation, whereas IL-12p40 was strongly increased (Fig. 3D, 3E). These data indicate that the kinase activity of MK2 transmits the p38α signal to stimulate mTOR signaling and to regulate the production of IL-12 and IL-10.

**p38 signals to mTOR via TSC1/TSC2 to regulate IL-12 and IL-10**

TSC1 and TSC2 act as dimer to inhibit activation of mTORC1 (29). To investigate whether p38-MK2 may mediate mTORC1 activation via TSC1/TSC2, we made use of MEFs deficient in either TSC1 or TSC2. Treatment with anisomycin or UV-stimulated p38 and mTOR signaling in Tsc1+/+ cells, as well as in Tsc2+/+ cells, and inhibition of p38 by either SB203580 or BIRB796 strongly reduced mTOR activation as shown by diminished phosphorylation of p70S6K or S6 (Fig. 4A–D). Strikingly, inhibition of p38 did not block mTOR signaling in cells deficient of TSC1 or TSC2 (Fig. 4A–D). Rapamycin, which acts downstream of TSC1/TSC2, still inhibited mTOR in these cells (Fig. 4A–D). These results suggest that the TSC1/TSC2 complex is a critical signaling node that senses p38 activity to regulate mTOR activation. Interestingly, serum, a well-known mTOR activator, did not stimulate p38 activation, and inhibition of p38 did not influence mTOR activation induced by serum (Fig. 4E). Previously, it has been shown that MK2 phosphorylates Ser1210 in TSC2 (37). However, it remained to be verified whether p38 activation regulates TSC2 activity via Ser1210 phosphorylation by MK2. To explore this possibility, we transfected Tsc2−/− cells with plasmids encoding either WT TSC2 or a S1210A TSC2 mutant and observed that WT, but not mutant, TSC2 restored the ability of anisomycin to activate p70S6K (Fig. 4F). Moreover, BIRB796 inhibited p70S6K activation upon p38 stimulation in cells transfected with WT, but not mutant, TSC2 (Fig. 4F). Overall, these results demonstrate that activation of p38 inhibits the TSC1/TSC2 complex, allowing mTOR activation potentially via MK2-dependent phosphorylation of TSC2 at Ser1210.

**FIGURE 4.** p38 signals to mTOR via TSC1/TSC2. (A and B) Tsc1+/+ and Tsc1−/− MEFs or (C–E) Tsc2+/+ and Tsc2−/− MEFs were starved overnight and treated with medium (−), BIRB796 (200 nM), SB203580 (2 μM), or rapamycin (Rap; 100 nM) for 90 min. Afterward, MEFs were stimulated with (A, C) anisomycin (100 ng/ml), (B, D) UV (1 min), or (E) 10% serum for 60 min. Whole-cell lysates were analyzed by immunoblotting. (F) Tsc2−/− MEFs were transfected with empty vector (e.v.), HA-tagged WT TSC2, or HA-tagged TSC2 S1210A and then treated with medium, BIRB796, and anisomycin as indicated. Immunoblotting was performed with the indicated Abs. Long exposure revealed the phosphorylation of the p85S6K (p85) isoform in addition to the p70S6K (p70) isoform. Densitometric analysis was performed on the intensity of p-p70S6K. One representative of three different experiments is shown. NB, Nonspecific bands.
p38α and PI3K independently regulate mTOR

Classical activation of mTOR signaling by growth factors or TLR ligands is dependent on PI3K (17, 38). Our results so far established that p38α activates mTOR via MK2 involving an Ser1210-dependent regulation of TSC2 to modulate IL-12/IL-10 signaling. To further delineate the relative requirements of PI3K and p38α for stimulating mTOR, we inhibited p38 in the presence or absence of wortmannin, a specific covalent PI3K inhibitor. Treatment with either wortmannin or BIRB796 considerably diminished phosphorylation of S6 in human LPS- or anisomycin-activated monocytes (Fig. 5A, 5B). The combination of both inhibitors led to a near-complete abolishment of S6 phosphorylation (Fig. 5A, 5B). Likewise, mTOR was blocked more efficiently in BMDMs from p38αD than from p38αfl/fl mice after wortmannin treatment (Fig. 5C, 5D). Immunofluorescence analysis confirmed that wortmannin inhibited the phosphorylation of S6 more completely in p38αD than in p38αfl/fl BMDMs (Fig. 5E), suggesting that PI3K and p38α cooperatively stimulate mTOR signaling. Next, we analyzed MEFs deficient in p85α/p85β, the regulatory subunits of PI3K, to assess the importance of p38 versus PI3K for maximum activation of mTOR (Fig. 5F). Phosphorylation of p70S6K and 4E-BP1 induced by LPS, anisomycin, or UV exposure was severely reduced but still detectable in p85−/− MEFs compared with their WT counterparts (Fig. 5F). However, concurrent inhibition of p38 by BIRB796 in p85−/− MEFs further inhibited activation of mTOR (Fig. 5F). Together, these results strongly suggest that full activation of mTOR in myeloid immune cells is dependent on p38 and PI3K.

**Discussion**

Monocytes, macrophages, and DCs are emerging therapeutic targets in cardiovascular, malignant, and autoimmune disorders. Addressing gaps in knowledge about the function of these cells in response to environmental stimuli is required to understand the in vivo responses to therapies that target these cells. Stringent control of MAPK signaling is critical for balancing proinflammatory versus anti-inflammatory signaling to enable efficient pathogen killing but also to limit detrimental tissue pathology. In that regard, p38 is one of the most studied drug targets for anti-

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**FIGURE 5.** p38α and PI3K independently regulate mTOR. (A and B) Human monocytes were incubated with medium (−), BIRB796 (200 nM), and/or wortmannin (WM; 100 nM) for 90 min and stimulated with (A) LPS (100 ng/ml) or (B) anisomycin (100 ng/ml). Cell lysates were analyzed by immunoblotting. (C–E) p38α−/− and p38αD BMDMs were treated with medium (−) BIRB796 (200 nM), SB203580 (2 μM), and/or wortmannin (WM; 100 nM) as indicated and stimulated with (C, E) LPS (100 ng/ml) or (D) anisomycin (100 ng/ml) for 1 h. (C, D) Cell lysates were analyzed by immunoblotting. (E) Phosphorylation of S6 and MK2 was analyzed by immunofluorescence; original magnification ×40. One representative experiment of three is shown. (F) p85−/− MEFS were treated with medium (−) or BIRB796 (200 nM) followed by stimulation with LPS (100 ng/ml), anisomycin (100 ng/ml), or UV (10 s) for 60 min. Cell lysates were analyzed by immunoblotting.
inflammatory therapy. This kinase directly or indirectly regulates many transcription factors and, therefore, participates in the gene induction of cytokines and other inflammatory molecules. p38 is also important in the posttranscriptional regulation of gene expression during inflammation (39). Animal studies have shown that p38 inhibitors are efficacious in several disease models, including inflammation, arthritis and other joint diseases, septic shock, and myocardial injury (11). However, translation into the clinic has been difficult either because of lack of efficiency or the appearance of adverse effects including inflammation such as skin rashes.

p38a is activated in diverse cell types by a wide array of stress stimuli including genotoxic agents, pathogen-associated molecular patterns, proinflammatory cytokines, heat or osmotic shock, oxygen partial pressure, or chemical insults (arsenite and anisomycin) (40). The simple view of an entirely proinflammatory kinase promoting the expression of TNF-α and IL-1β shifted to a more complex role in recent years demonstrating that p38a controls IL-12 and IL-10 expression (14–16). However, the downstream pathway that regulates these immunomodulatory cytokines remained unknown.

In this article, we have demonstrated that p38a uses the TSC2/mTOR signaling pathway to control the balance of IL-12 and IL-10. Our data suggest that TLR ligands or stress stimuli lead to an activation of p38a that, in turn, activates its downstream kinase MK2. The kinase activity of MK2 most likely phosphorylates Ser1210 of TSC2, leading to inactivation of the TSC1/TSC2 complex and, in turn, activation of the mTOR pathway (Fig. 6C). Activation of mTOR then promotes IL-10 production, whereas reducing IL-12 expression. Our work indicates that p38a-mediated mTOR activation occurs in parallel to the well-known PI3K pathway that activates mTOR in response to TLR signals (17, 19). Hence, both pathways concurrently control mTOR activation to precisely allow the expression of proinflammatory and anti-inflammatory cytokines in response to environmental stress. We are unaware of a report demonstrating that two stimuli additively regulate the activation of mTOR via the TSC complex. We suggest that p38a-mediated mTOR activation in addition to the PI3K pathway represents a tuning mechanism to regulate immunomodulatory cytokines to adapt the immune response to the environmental milieu. This is supported by the observation that hyperactivation of p38a by anisomycin can modulate IL-12 and IL-10 expression on top of a TLR signal (Fig. 2D). The p38/MK2 axis is required after excessive tissue damage to induce tissue repair (41, 42). In such situations, p38a may promote mTOR activation in resident and recruited macrophages to reduce IL-12 and augment IL-10 production that limits the generation of a proinflammatory CD4+ Th1 response that would further exaggerate tissue damage (43).

A link from p38β to mTOR has been described in Drosophila that occurs via a TSC2-independent mechanism (44). In line, p38β was recently shown to phosphorylate the essential mTORC1 binding protein Raptor and to participate in arsenite-induced mTOR activation in fibroblasts (45). In contrast, in the same cell, p38β can also inhibit mTOR upon energy starvation via phosphorylation and inactivation of Ras homolog enriched in brain (Rheb), a key component of the mTORC1 pathway (46). We now show that p38a via MK2 promotes mTOR activation dependent on the TSC1/TSC2 complex in myeloid immune cells. Indeed, MK2 was shown to phosphorylate TSC2 at Ser1210 in fibroblasts (37). In addition, MK2 was also described to phosphorylate Akt at Ser473 in neutrophils (47), in line with the inhibition of Akt Ser473 by the p38 inhibitors in our cells (Figs. 2A, 6). We have previously shown that mTOR regulates NF-κB and STAT3 signaling (24). Interestingly, p38α and MK2 also are required for STAT3 activation and IL-10 production (14, 36). These

**FIGURE 6.** p38a and PI3K coordinate regulation of IL-12/IL-10 balance. (A) p38αβ−/− and p38α−/− MDMs were treated with wortmannin (WM; 100 nM) and stimulated with medium (−). SAC (75 μg/ml), or LPS (100 ng/ml) for 24 h. Cytokine levels in cell-free supernatants were determined by Luminex (means ± SEM; n = 5). (B) Human monocytes were treated with wortmannin (WM; 100 nM) or BIRB796 (200 nM) as indicated and stimulated with LPS (100 ng/ml) for 24 h. IL-12p40 and IL-10 were determined in the supernatants by Luminex (means ± SEM; n = 3). (C) Model of mTOR-regulated production of IL-12/IL-10 via PI3K and p38 on the level of TSC1/TSC2. *p < 0.05; n.s., Not significant.
effects are likely to be indirectly mediated by p38 and mTOR, and the precise downstream pathways how mTOR regulates IL-12 versus IL-10 needs further investigation. In this study, we focused on delineating the mechanism of p38-dependent mTOR regulation in myeloid immune cells.

Rapamycin is currently evaluated as vaccine adjuvant, especially because of its ability to enhance memory CD8+ T cell responses. In addition, previous work also established that rapamycin exerts immunostimulatory effects via the innate immune system that may contribute to the adjuvant properties of rapamycin (23–25, 48, 49). However, its immunosuppressive activity will likely prevent the inclusion of rapamycin in widely distributed vaccines. Our data suggest that inhibition of p38 might be similarly effective as adjuvant strategy where strong Th1 responses are desired, and moreover, it might avoid the potent immunosuppressive effects on the T cell compartment as p38 is regarded dispensable for T cell function (50). Indeed, in a mouse model of Listeria monocytogenes infection, vaccination with SB203580 was protective by inducing efficient Th1 immunity (16).

In summary, we have identified and characterized a pathway from p38α to mTOR via MK2 and TSC1/TSC2 in myeloid immune cells that tunes the immune response according to environmental input signals.

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Disclosures

The authors have no financial conflicts of interest.

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