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*J Immunol* 2003; 171:4792-4800; doi: 10.4049/jimmunol.171.9.4792

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Novel and Detrimental Effects of Lipopolysaccharide on In Vitro Generation of Immature Dendritic Cells: Involvement of Mitogen-Activated Protein Kinase p38

Jin Xie,* Jianfei Qian,* Siqing Wang,* Muta E. Freeman III,* Joshua Epstein,*† and Qing Yi2*†

Dendritic cells (DCs) are recognized as major players in the regulation of immune responses to a variety of Ags, including bacterial agents. LPS, a Gram-negative bacterial cell wall component, has been shown to fully activate DCs both in vitro and in vivo. LPS-induced DC maturation involves activation of p38, extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinases, and NF-κB. Blocking p38 inhibits LPS-induced maturation of DCs. In this study we investigated the role of LPS in the in vitro generation of immature DCs. We report here that in contrast to the observed beneficial effects on DCs, the presence of LPS in monocyte culture retarded the generation of immature DCs. LPS not only impaired the morphology and reduced the yields of the cultured cells, but also inhibited the up-regulation of surface expression of CD1a, costimulatory and adhesion molecules. Furthermore, LPS up-regulated the secretion of IL-1β, IL-6, IL-8, IL-10, and TNF-α; reduced Ag presentation capacity; and inhibited phosphorylation of ERK, but activated p38, leading to a reduced NF-κB activity in treated cells. Neutralizing Ab against IL-10, but not other cytokines, partially blocked the effects of LPS. Inhibiting p38 (by inhibitor SB203580) restored the morphology, phenotype, and Ag presentation capacity of LPS-treated cells. SB203580 also inhibited LPS-induced production of IL-1β, IL-10, and TNF-α; enhanced IL-12 production; and recovered the activity of ERK and NF-κB. Thus, our study reveals that LPS has dual effects on DCs that are biologically important: activating existing DCs to initiate an immune response, and inhibiting the generation of new DCs to limit such a response. The Journal of Immunology, 2003, 171: 4792–4800.

Dendritic cells (DCs) are the sentinels of the immune system (1, 2). In their immature state DCs reside in peripheral tissues, where they survey for incoming pathogens. Encounter with pathogens leads to DC activation and migration to secondary lymphoid organs where they trigger a specific T cell response. DCs are also the most potent APCs; they are not only the cells that can stimulate quiescent, naive CD4+ and CD8+ T cells and B cells and initiate primary immune responses, but they can also induce a strong secondary immune response with relatively small numbers of DCs and low levels of Ag. Furthermore, DCs are involved in polarization of the T cell response via secreted cytokines and induction of tolerance through deletion of self-reactive thymocytes and anergy of mature T cells (2). Given their central role in controlling immunity, DCs are logical targets for many clinical situations that involve T cells, such as transplantation, allergy, autoimmune disease, resistance to infections and tumors, immunodeficiency, and vaccination.

The maturation process is central to the function of the DCs and enables one cell to perform different, highly specialized functions sequentially. There are many stimuli that can initiate this maturation process in vitro. These include the proinflammatory cytokines TNF-α and IL-1β, ligation of CD40 by CD40 ligand or anti-CD40 Abs, and bacterial products (2–4). LPS, a Gram-negative bacterial cell wall component, has been shown to fully activate DCs both in vitro and in vivo. The activation of DC by LPS or LPS-induced inflammatory products up-regulates the expression of surface MHC class I and II, costimulatory and adhesion molecules CD40, CD54, CD80, and CD86, and eventually results in the development of an Ag-specific T cell response (5, 6). Although significant progress has been made over the past several years (7–10), signal transduction pathways involved in (LPS-induced) maturation of DCs are still poorly characterized. It has been shown that LPS interacts with immature DCs through the surface receptor Toll-like receptor-4 (TLR-4), leading to activation of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinases (MAPKs), and NF-κB (7, 8) and production of IL-12 (4, 11). Blocking NF-κB or MAPK p38 inhibits LPS-induced maturation of DCs, indicating that NF-κB and p38 MAPK play pivotal roles in the process.

Although LPS has been identified as an activation and maturation agent for DCs, its role in the differentiation and generation of immature DCs from their precursor cells has not been well studied (12, 13). The establishment of in vitro culture systems, allowing the induction of human DCs from various precursors, offers the possibility to study molecular mechanisms involved in DC differentiation. This study investigated the effects of LPS on the generation of immature, monocyte-derived DCs (MoDCs) and the molecular events that accompany this. Like DCs, monocytes express LPS receptor TLR-4 (14) and, in addition, CD14 (15), both of which are able to interact with LPS. Furthermore, it has been

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Flow cytometric analysis

Cells harvested on days 7 and 9 were analyzed for their surface expression of relevant molecules. This was conducted using a FACScan (BD Biosciences, San Jose, CA). Briefly, cells were first washed twice in PBS, followed by addition of PE- and FITC-conjugated mAbs. After incubation on ice for 30 min the cells were washed twice, resuspended in PBS, and prepared for analysis. Controls consisted of cells stained with irrelevant mouse IgG Abs.

Measurement of cytokines by cytokimetric bead array analysis

Kits of cytokimetric bead array analysis for the detection of cytokines (18), including IL-1β, IL-6, IL-8, IL-10, IL-12, and TNF-α (inflammatory cytokine kit), and IL-2, IL-4, IL-5, IL-10, IFN-γ, and TNF-α (T cell subset cytokine kit), were purchased from BD PharMingen, and assay was performed by the Core Facility at Department of Microbiology and Immunology, University of Arkansas for Medical Sciences. Briefly, supernatants of monocyte or T cell culture were collected and kept frozen at −80°C until analysis. When assayed, the supernatants were mixed with human cytokine-captured beads, and PE-conjugated detection reagent was added and incubated for 3 h at room temperature. After the incubation, the beads were washed three times, resuspended in PBS, and prepared for flow cytometric analysis.

Endocytic activity assay

To evaluate the capacity for uptake via mannose receptor-mediated endocytosis, cultured cells or MoDCs were incubated with 1 mg/ml FITC-dextran at 37 or at 0°C (control for autofluorescence intensity) for 1 h (8, 12) and then washed four times with ice-cold PBS. Cells were analyzed by flow cytometer.

Allogeneic MLR assay

To examine the capacity of MoDCs or LPS-treated cells to activate allogeneic T cells, an allogeneic MLR was used. Briefly, purified allogeneic T cells (8 × 10^5 cells/100 μl/well) were seeded in 96-well U-bottom tissue culture plates. MoDCs or LPS-treated cells in various numbers were added and cultured at 37°C in 5% CO₂ for 6 days. Sixteen hours before harvest, 1 μCi of [³H]thymidine was added to each well. Cells were harvested, and radioactivity was measured in a beta-lux scintillation analyzer (Packard, Meriden, CT). Results are expressed as the mean counts per minute of triplicate cultures.

Presentation of soluble Ag by MoDCs

To examine the capacity of MoDCs and LPS-treated cells to capture and present soluble Ag and to activate autologous Ag-specific T cells, an assay of the T cell response to recall Ag PPD was performed. This was conducted with cells from healthy blood donors who had been immunized with bacillus Calmette-Guérin vaccines and showed a positive T cell proliferative response against PPD in vitro. The cultured cells were pulsed with 2.5 μg/ml of PPD for 3 times for 2 h, washed three times with PBS, and cultured with purified autologous T cells for 6 days. The T cell proliferative response was measured by overnight incubation with [³H]thymidine (1.0 μCi/well), as described for the MLR assay.

Western blot analysis

To detect intracellular signaling associated with LPS treatment, Western blots were used to analyze MAPKs, NF-κB, and STAT3 expression by cultured cells. To detect MAPKs, IκBα, and IκBβ, cells were cultured with or without LPS, harvested, washed, and lysed with lysis buffer (50 mM Tris...
(pH 7.5), 140 mM NaCl, 5 mM EDTA, 5 mM Na$_3$N$_3$, 1% Triton X-100, 1% Nonidet P-40, and 1× protease inhibitor cocktail). For the determination of phosphorylated MAPKs, IκBα, and STAT3 (Tyr705), cells were lysed directly in Laemmli buffer (Sigma-Aldrich). The samples were centrifuged at 12,000 × g, and the supernatants were collected, boiled, and subjected to SDS-PAGE. After transfer to nitrocellulose membrane and subsequent blocking, the membranes were immunoblotted with respective Abs against phosphorylated MAPKs, IκB, or STAT3 and visualized with alkaline phosphatase-conjugated secondary Abs, followed by enhanced chemiluminescence (Bio-Rad Laboratories, Hercules, CA). For protein quantification, blots were scanned and analyzed by spot densitometry using the Alphalmager 2200 Documentation and Analysis System (Innotech, San Leandro, CA). The results are expressed as the average value of pixels enclosed (AVG). AVG is calculated as the sum of all pixel values after background correction divided by area.

Statistical analysis
Student’s $t$ test was used to compare various experimental groups; significance was set at $p < 0.05$.

Results
Detrimental effects of LPS on the generation of immature MoDCs
To examine its effects on in vitro generation of immature MoDCs, LPS was added at the beginning of cell culture at a low dose (1 ng/ml) that mimics the blood endotoxin concentrations seen in severe sepsis (19) and at a high dose (1 μg/ml) that has been the commonly used dose to induce DC maturation in vitro (13, 20). In all experiments LPS was continuously present in the cultures, although its concentrations were gradually decreased due to medium change every 2 days without further addition of the endotoxin. Fig. 1 depicts the morphology of control MoDCs and LPS-treated cells cultured for 7 days in medium with or without addition of LPS. It is evident that the presence of endotoxin in culture medium affected cell morphology; cells cultured with LPS at both doses were smaller, with poorly differentiated, macrophage-like morphology, while cells cultured in medium without LPS appeared as mostly floating, single cells with enlarged, DC-like morphology. In addition, a lower cell yield, determined by viable large cell count, was obtained in cultures with LPS ($p < 0.05$ for both concentrations; Fig. 2A). We next examined whether induction of apoptosis by LPS was responsible for the low cell yield. It was analyzed using flow cytometric analysis with FITC-conjugated annexin V and propidium iodide. No difference was observed between the cultures with or without LPS (data not shown), suggesting that LPS did not induce apoptosis in treated cells; instead, fewer cells were differentiated into large, immature MoDCs in the presence of LPS. Thus, cells recovered from the cultures with the addition of LPS are referred to in this study as LPS-treated cells, not as MoDCs. In

FIGURE 2. LPS reduced cell yields (big cell population) and up-regulated the secretion of proinflammatory cytokines by cultured cells. A, Yields of cultured cells, determined by viable cell counts of big cells only, from day 7 cultures with or without addition of 1 ng/ml and 1 μg/ml of LPS. Data are expressed as a percentage of the control. B and C, Cytokine secretion by cells on day 3 from cultures with or without the addition of 1 ng/ml and 1 μg/ml of LPS or with 1 μg/ml of LPS and 10 μM SB203580 (LPS+SB). The data shown are the mean ± SEM from three independent experiments.
these and the following studies, PBMCs from five healthy blood donors were used to generate immature MoDCs.

DCs are the most potent APCs; they are not only required for the priming of native CD4+ and CD8+ T cells to initiate an immune response, but also play an active role in polarizing the immune response toward either type 1 or type 2 T cell responses (2). Cytokines secreted by DCs are instrumental to the process. Therefore, we examined whether LPS could influence the cytokine secretion profile of treated cells. In these experiments highly purified monocytes were used to generate immature MoDCs. Supernatants were collected from cultured cells on day 3, and a flow cytometry-based bead array analysis was employed to measure the relevant (inflammatory) cytokines. As evident by the results depicted in Fig. 2, B and C, LPS treatment significantly up-regulated the secretion of IL-1β (p < 0.01), IL-6 (p < 0.01), IL-8 (p < 0.01), IL-10 (p < 0.01), and TNF-α (p < 0.05) by cultured cells. The concentration of IL-12 was low (~1 pg/ml) in medium and was slightly up-regulated by the addition of LPS (IL-12, 3-9 pg/ml; p < 0.05).

We next examined the surface expression of DC-related molecules on cultured cells. After 7-day culture, cells were washed three times and stained with PE- and FITC-conjugated Abs. As shown by the representative histograms of flow cytometric analysis of cultured cells (Fig. 3), surface expression of CD1a, CD40, CD54, CD80, and CD86 was significantly (1.4- to 2.3-fold; p < 0.05 and p < 0.01) lower on 1 μg/ml LPS-treated cells. The effects of LPS at 1 ng/ml on these cells were similar, but less pronounced (data not shown). The expression of HLA-ABC and -DR on LPS-treated cells was also lower, but not significantly different from that on control MoDCs.

The results to date indicate that addition of LPS impaired the morphology and phenotype and altered the cytokine secretion profiles of cultured cells, which may lead to an abnormal function of these cells. Thus, we investigated whether the Ag presentation capacity of LPS-treated cells was different from that of normal immature MoDCs. First, FITC-dextran was used to examine the Ag uptake capacity (at 37°C) of the cells, because immature DCs have the highest capacity to capture and take up particular Ags via macropinocytosis and binding to mannose receptor. As shown in Fig. 4A, LPS at the high, but not the low, concentrations significantly reduced the uptake of FITC-dextran (p < 0.05). Cells incubated with FITC-dextran at 0°C could not phagocytose the particles and thus served as fluorescence controls. Second, using an allogeneic MLR assay, our results show that LPS-treated cells had a reduced capacity to activate alloreactive T cells (p < 0.05 and p < 0.01; Fig. 4B). The defect was more pronounced in cells treated with 1 μg/ml of LPS (p < 0.01). Furthermore, LPS-treated cells were also poor at presenting recall Ag PPD to and activating autologous, PPD-specific T cells (Fig. 4C; p < 0.01). Collectively, these data indicate that the function of cultured cells was severely impaired by LPS treatment.

LPS activated MAPK p38, but inhibited ERK, leading to reduced NF-κB and higher STAT3 activity in treated cells

As MAPKs play a critical role in the regulation of cell growth and differentiation, and NF-κB and STAT3 are involved in the regulation of cytokine expression or response to cytokines such as IL-6 and IL-10, we examined the levels and activity of these molecules by Western blot analysis. Cell lysates collected on days 1, 3, and 5 of the cultures were prepared for the analyses. Highly purified monocytes were used in these experiments to generate immature MoDCs. In this study the expression of MAPK family members, i.e., the phosphorylated ERK, p38 MAPK, and stress-activated protein kinase/JNK was examined, as were the nonphosphorylated MAPKs, which could serve as controls for protein loading. As shown in Fig. 5, compared with controls, significantly higher levels of phosphorylated p38 (pp38) were observed in cells treated LPS (at both 1 ng/ml and 1 μg/ml) on days 1 and 3 (p < 0.01 and p < 0.05; Fig. 5, A and B), which gradually declined thereafter. In contrast, a lower level of phosphorylated ERK1 and ERK2 (pERK) was observed in cells treated with LPS on days 1 and 3 (p < 0.01 and p < 0.05), but it gradually increased in treated cells thereafter (Fig. 5, A and C). During culture the levels of pERK were high and remained stable in control cells. The levels of nonphosphorylated p38, ERK, and MAPK kinase (MEK) remained stable (Fig. 5A). No changes were observed in the expression of stress-activated protein kinase/JNK (data not shown). These findings indicate that while monocytes cultured in the presence of IL-4 and GM-CSF expressed a relatively high level of pERK and a low level of p38, the addition of LPS to the culture up-regulated the expression of p38 and down-regulated that of pERK. As the exogenous LPS was consumed or diluted, because no additional endotoxin was added during the culture when 50% of the medium was replaced every 2 days, the expression of pp38 and pERK gradually returned to the levels seen in control cells.

For the detection of STAT3 activity, we used an Ab to detect phosphorylated STAT3 (pSTAT3) at Tyr705, which is an activated form of the molecule. As shown in Fig. 5, A and D, a significantly higher level of pSTAT3 was seen in cells treated with LPS on days 1, 3, and 5 (p < 0.01). Little or no STAT3 activity was detected in cells cultured in medium without the addition of LPS. No significant difference was observed in the expression of nonphosphorylated STAT3 protein between cells or in the same cells during

![FIGURE 3](http://www.jimmunol.org/)

**FIGURE 3.** Phenotype of immature MoDCs and LPS-treated cells. Cells were harvested on day 7 from cultures with or without the addition of 1 μg/ml of LPS or with LPS and SB203580 and were stained with Abs against DC-related surface markers (shaded histograms). Open histograms represent control Ab staining of the cells. Representative histograms of five independent experiments are shown.
culture (Fig. 5A). These results indicate that addition of LPS to the culture activated STAT3.

To detect NF-κB activity, we examined the expression or activity of its inhibitors, IκBα, IκBβ, and phosphorylated IκBα (pIκBα), since phosphorylation of serine residues on the IκBα proteins (appeared as elevated levels of pIκB) by kinases marks them for destruction via the ubiquitination pathway, thereby allowing activation of the NF-κB complex (16). Compared with those in medium only, cells cultured in the presence of LPS had higher levels of pIκBα (Fig. 5, A and E), but lower levels of IκBα (Fig. 5, A and F) on day 1, which gradually decreased (pIκBα) or increased (IκBα), respectively, in cells treated with 1 μg/ml of LPS during culture. This suggests that NF-κB activity was high in LPS-treated cells on day 1, but declined thereafter. In contrast, the levels of pIκBα were lower, and those of IκBα were higher in control cells on day 1; during culture the levels of pIκBα gradually increased, and those of IκBα decreased, respectively, suggesting an enhanced activity of NF-κB during cell differentiation induced by IL-4 and GM-CSF. The level of IκBβ remained stable (Fig. 5A). Collectively, these findings indicate that while NF-κB activity was low in starting monocytes and was up-regulated gradually during cell differentiation to immature MoDCs, its activity in LPS-treated cells was high initially, but decreased thereafter.

Neutralizing IL-10 partially blocked the effects of LPS on the generation of immature MoDCs

The results to date demonstrate that LPS impaired in vitro generation of immature MoDCs. It was conceivable that the inflammatory cytokines, such as IL-6, TNF-α, and IL-10, and MAPKs, especially p38, were involved. To examine the effects of these cytokines on the generation of immature MoDCs, neutralizing Abs against IL-6, TNF-α, and IL-10 were used. It appeared that neutralizing Ab against IL-10, but not to the other two cytokines, had a minor effect, as addition of the Ab (20 μg/ml) to culture partially restored LPS-inhibited surface expression of DC-related molecules, such as CD1a and CD54 (Fig. 6). However, all these Abs were potent at neutralizing their respective cytokines, as revealed by the cytometric bead array analysis (data not shown). The combination of these neutralizing Abs had no synergistic effects (data not shown). In line with these results, addition of exogenous IL-10 (10 ng/ml), but not the other cytokines, to the culture also impaired, although to a lesser extent, the generation of immature MoDCs (data not shown). Thus, these observations indicate that IL-10 plays a role in the LPS-induced alterations seen in treated cells.
The p38 inhibitor restored the generation of functional immature MoDCs

We next examined the importance of p38 in LPS-induced alterations in DCs. A p38 inhibitor, SB203580, was chosen, which was added (10 μM) to the cells at the beginning of the culture. In these and the following experiments LPS at 1 μg/ml was used to retard the generation of immature MoDCs. Our results show that inhibition of p38 MAPK in LPS-treated cells not only restored the morphology (Fig. 1) and phenotype (Fig. 3) of the cultured cells, but also significantly inhibited the up-regulation of IL-1β (p < 0.05), TNF-α (p < 0.01), and IL-10 (p < 0.01), but not IL-8, production induced by LPS (Fig. 2, B and C). Interestingly, the inhibitor SB203580 further up-regulated IL-12 (6-fold; p < 0.01) and IL-6 (1.2-fold; p > 0.05) production by the cells in response to LPS (Fig. 2 B and C).

Inhibition of p38 MAPK in LPS-treated cells also enhanced their Ag uptake and presentation capacity. As shown in Fig. 7A, SB203580 treatment completely restored FITC-dextran uptake by LPS-treated cells. In addition, these cells had the same capacity to stimulate allospecific T cells (Fig. 7B) and to present recall Ag PPD and activate PPD-specific, autologous T cells (Fig. 7C) as the control MoDCs. Hence, inhibition of p38 MAPK restored not only the morphology and phenotype, but also the function of LPS-treated cells. These results confirm that p38 MAPKs play a crucial role in LPS-induced retardation in the generation of functional MoDCs.

Western blot analysis revealed that inhibition of p38 MAPK in LPS-treated cells was accompanied by changes in cell signaling molecules. As it has been recognized that the p38 MAPK inhibitor SB203580 binds to the ATP-binding pocket of p38 kinase and inhibits its activity, but does not prevent it from being phosphorylated (8, 21), a strong band of pp38 was still seen in cells treated with both LPS and SB203580 (Fig. 8A). However, addition of SB203580 to the culture effectively abolished LPS-induced inhibition of ERK phosphorylation and activation (Fig. 8, A and B). The levels of nonphosphorylated ERK remained unchanged. No difference was observed in the expression of STAT3 and pSTAT3. Furthermore, SB203580 up-regulated the expression of pIκBα and down-regulated that of IκBα (Fig. 8, A and B), indicating that inhibition of p38 led to enhanced NF-κB activity in LPS-treated cells. The level of IκBβ remained stable. Taken together, these

FIGURE 5. Western blot analysis of LPS-induced signaling. A, Western blots of pp38, ERK (pERK), STAT3 (pSTAT3), and IκBα (pIκBα). The nonphosphorylated kinases and proteins (p38, ERK, STAT3, IκBα, and IκBβ) were also analyzed and serve as the control for protein loading. Cells were cultured in medium (lanes 1, 4, and 7) or with the addition of 1 ng/ml (lanes 2, 5, and 8) and 1 μg/ml (lanes 3, 6, and 9) of LPS. Representative blots from three independent experiments are shown. Densitometric data (AVG; mean ± SEM of three independent experiments) for pp38 (B), pERK (C), pSTAT3 (D), pIκBα (E), and IκBα (F) are also shown.

FIGURE 6. Blocking endocrine IL-10 partially inhibited the effects of LPS on the generation of immature MoDCs. Neutralization Ab against IL-10 (20 μg/ml) was added to cultures with LPS (1 μg/ml), and cells were harvested on day 7 and analyzed for their phenotype. Shown are cells stained with Abs against CD1a and CD54 (shaded histograms). Open histograms represent control Ab staining of the cells. Representative histograms of five independent experiments are shown.
findings suggest that LPS binding to cell surface receptors activates p38, which inactivates the (Raf/MEK)/ERK MARK pathway, leading to reduced NF-κB activity, and impaired the differentiation of monocytes to immature MoDCs. Inhibiting p38 activity overcomes the negative effects of LPS on the cells.

**Defective maturation of LPS-pretreated cells**

We wondered whether maturation of cultured cells with LPS or cytokines TNF-α and IL-1β for an additional 48 h could correct the defects caused by LPS treatment. In these experiments immature MoDCs or cultured cells were generated in medium with or without addition of LPS at 1 μg/ml. On day 7 cells were washed, and LPS (1 μg/ml) or recombinant TNF-α and IL-1β (5, 17) were added to induce DC maturation. After 48 h cells were harvested and analyzed for their phenotype. As shown in Fig. 9, cells cultured in the absence of LPS for the first 7 days significantly (4- to 32-fold) up-regulated, compared with control immature MoDCs, the expression of CD40, CD83, CD80, CD86, and MHC class I and II molecules after 48-h culture with LPS, while cells cultured in the presence of LPS for the first 7 days displayed poor morphology (data not shown) and phenotype after maturation. Addition of the p38 inhibitor SB203580 to the culture restored, to a large extent, the phenotype of a mature DC. Similar effects were obtained with TNF-α and IL-1β on mature DCs (data

**FIGURE 8.** The p38 inhibitor SB203580 recovered ERK and NF-κB activity in LPS-treated cells. A. Western blots of phosphorylated p38 (pp38), ERK (pERK), STAT3 (pSTAT3), and IκBα (pIκBα). The nonphosphorylated kinases and proteins (p38, ERK, STAT3, IκBα, and IκBβ) were also analyzed and serve as the control for protein loading. Representative blots from four independent experiments are shown. B. Densitometric data (AVG; mean ± SEM of four independent experiments) for pp38, pERK, pSTAT3, pIκBα, and IκBα are also shown.
not shown). Thus, these results indicate that the damage caused by LPS during the differentiation of monocytes remained after maturation.

**Discussion**

Although it is well documented that LPS is a potent activator of DCs in vitro and in vivo, its role in the differentiation of immature DCs from their precursors has not been examined. The goal of the present study was to investigate whether LPS could affect in vitro generation of immature DCs from peripheral blood monocytes, as monocytes express LPS receptors TLR-4 and CD14 and therefore are sensitive to LPS stimulation. Moreover, it has been shown that a portion of lymph node DCs is derived from monocytes in vivo (16). Thus, such an in vitro study is biologically relevant and important. In our experiments LPS at a low concentration (1 ng/ml), which is within the range of blood endotoxin concentrations seen in severe sepsis, and a high concentration (1 μg/ml) is commonly used to induce DC maturation in vitro, were used and added to monocytes at the beginning of the culture. Cells were then cultured, with or without the continuous presence of LPS, in medium supplemented with GM-CSF and IL-4. On day 7 cells were collected and subjected to tests. Our results showed that in contrast to the observed activating effects on DCs, LPS (at both concentrations) retarded the generation of immature MoDCs. LPS reduced the yields of cultured cells, impaired cell morphology, and inhibited the up-regulation of CD1a, CD40, CD54, CD80, and CD86 expression on treated cells. The expression of MHC class I and II Ags, although not significantly, was also lower on LPS-treated cells compared with control MoDCs. In addition, LPS stimulated cultured cells to secrete significantly higher amounts of proinflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α and the immunosuppressive cytokine IL-10 and reduced the cells’ capacity to take up particulate Ags, activate allospecific T cells, present recall Ag PPD, and activate autologous, PPD-specific T cells. These defects were sustained after an additional 48-h culture with LPS or with TNF-α and IL-1β to induce maturation of these cells. Collectively, these findings indicate that LPS is detrimental to the differentiation of immature DCs from blood monocytes. Thus, our study identifies LPS as a negative regulator of DC differentiation. Further study is needed to examine whether LPS could also affect the differentiation of immature DCs from CD34+ stem cells.

DCs are professional APCs that represent the essential link between innate and acquired immunity (2). DCs express different TLRs that function as pattern recognition receptors specific for microbial pathogens, such as LPS, lipoproteins, and bacterial DNA (14, 22, 23). During infection, signaling through TLRs triggers DC maturation, which results in the increase in surface expression of MHC class I and II, costimulatory and adhesion molecules and the production of numerous proinflammatory cytokines that are essential for initiating T cell-mediated immune responses. On the other hand, our study demonstrates that in the presence of LPS the differentiation and generation of immature MoDCs were retarded, indicating that LPS also has a negative effect on DCs or their precursor cells. Conceivably, both effects are biologically important and required. By activating existing DCs, LPS is able to facilitate the generation of a beneficial antibacterial immune response to eliminate the infection, and by inhibiting the generation of new immature DCs, it can also limit the scale of the immune response to minimize damage to normal tissue. Hence, LPS can act as both positive and negative regulators of the immune system.

The signaling pathways involved in LPS-induced DC maturation seem to differ from those involved in LPS-induced retardation of DC precursor differentiation. It is known that interaction of LPS with TLR-4 on immature DCs leads to phosphorylation and activation of all three MAPKs, i.e., ERK, JNK, and p38, and NF-κB (7–10). Blocking NF-κB or p38 inhibits DC maturation induced by LPS, indicating that activation of p38 MARK and NF-κB is essential for DC maturation (8, 24, 25). In our study, however, the interaction of LPS with its surface receptors on monocytes activated MAPK p38, but reduced the expression of phosphorylated ERK. No change was noted with SAK/JNK. In addition, such an interaction led to a reduced activity of NF-κB, but activated STAT3 in treated cells. These results suggest that LPS selectively activates p38, but inhibits ERK and NF-κB activity in treated cells. As NF-κB is a common transcription factor of the Raf/MEK/ERK pathway (26, 27), it is conceivable that the reduced NF-κB activity in LPS-treated cells was the result of a suppressed Raf/MEK/ERK cascade. Alternatively or concomitantly, the increased secretion of IL-10 by the treated cells could also be responsible for the decreased ERK and NF-κB activities (28). As NF-κB plays a pivotal role in DC differentiation, maturation, and function (27), it is plausible that the reduced NF-κB activity was responsible for the retarded DC differentiation induced by LPS.
Furthermore, the p38 inhibitor restored the activity of ERK and MAPK p38 and the secretion of IL-10, and the inactivation of ERK of immature MoDCs, which was dependent on the activation of the bacterial endotoxin to monocyte culture retarded the generation of new DCs so that the scale of the immune response is reduced. Thus, our study reveals that LPS has dual effects on DCs that both completely abrogate the effects of LPS on generation of the cells. These findings suggest that activation of p38 may be detrimental to DC growth, differentiation and survival signals relayed through the IL-6 family of growth, differentiation and survival signals. 

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