*International Immunology, Vol. 16, No. 2, pp. 1701–1709*
doi:10.1093/intimm/dxh171 Advance Access publication 11 October 2004


# Role of c-Jun N-terminal kinase on lipopolysaccharide induced maturation of human monocyte-derived dendritic cells

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*Keywords*: cytokines, mitogen-activated protein kinases, signal transduction, Th1/2 cells

**Abstract**

**Dendritic cells (DCs) are potent antigen-presenting cells that play a pivotal role in the initiation of T cell-dependent immune responses. Immature DCs obtained from peripheral blood $CD14^+$ monocytes by culture with granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) differentiate into mature DCs upon stimulation with lipopolysaccharide (LPS). At least three families of mitogen-activated protein kinases (MAPKs), that is, extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK) and p38 MAPK, are involved in the DC maturation process. We report investigations of the role of JNK in the maturation of human monocyte-derived DCs. SP600125, a specific inhibitor of JNK, inhibited the LPS-induced up-regulation of CD80, CD83, CD86 and CD54, but augmented the up-regulation of HLA-DR. SP600125 slightly inhibited the down-regulation of FITC–dextran uptake during DC maturation. However, SP600125 did not affect the LPS induced up-regulation of allostimulatory capacity of DCs. SP600125 inhibited the release of IL-12 p70 and TNF-α from mature DCs. Although autologous T cells primed by the ovalbumin (OVA)-pulsed mature DCs produced IFN-γ, but not IL-4, OVA-pulsed SP600125-treated mature DCs could initiate IL-4 production from autologous T cells. In contrast, a p38 MAPK inhibitor, SB203580, profoundly inhibited the phenotypic and functional maturation of DCs, while an ERK inhibitor, PD98059, had little or no effect. Taken together, the JNK signaling pathway appears to have a role that is distinct from the p38 MAPK and ERK cascades in the maturation process of DCs, and may be involved in the augmentation of Th2-prone T cell responses when it is suppressed.**

## Introduction

Dendritic cells (DCs) are potent antigen-presenting cells that can stimulate resting T cells in the primary immune response. Immature DCs reside in almost all tissues, where they can capture and process antigens (1,2). Thereafter, they migrate toward the T-cell areas of secondary lymphoid organs via the afferent lymphatics. During the migration, they lose their ability to internalize antigens and acquire the capacity to present antigens to naive T cells, a process referred to as DC maturation or activation (3,4). Fully mature DCs show high surface expression of both costimulatory and adhesion molecules such as CD40, CD54, CD80 and CD86, as well as MHC class II antigens, but have a decreased capacity for antigen uptake and processing. Up-regulation of CD83, a specific marker for DC maturation, also occurs (3–6). The maturation process *in vitro* can be initiated by inflammatory stimuli such as tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β), components of infectious agents such as lipopolysaccharide (LPS) and unmethylated DNA CpG motif, CD40 ligation and contact sensitizers (7–10).

DCs can be induced from $CD34^+$ cells in human cord blood and bone marrow in the presence of granulocyte macrophage-colony stimulating factor (GM-CSF) and TNF-α or by culturing peripheral blood $CD14^+$ monocytes with GM-CSF and IL-4 (3,11,12). Immature monocyte-derived DCs further differentiate into mature DCs upon stimulation with LPS (7). These culture systems are useful for the study of molecular mechanisms and signal transduction pathways involved in DC maturation. At least three distinct mitogen-activated protein kinase (MAPK) signaling cascades exist in mammals, including the extracellular signal-regulated kinases (ERK), the p38 MAPK and the c-Jun N-terminal kinases (JNK) pathways (13–15). The availability of specific inhibitors for the ERK and

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*Transmitting editor*: S. Koyasu

*Received* 18 May 2004, *accepted* 13 September 2004

p38 MAPK pathways allows the evaluation of their involvement in the human DC maturation process (16–18). A p38 MAPK inhibitor, SB203580, has been reported to inhibit both the allostimulatory function and cell surface expression of co-stimulatory and adhesion molecules (16). In contrast, an ERK inhibitor, PD98059, potently inhibits TNF-α production but does not inhibit either the immunostimulatory function or cell surface expression of CD80, CD83 and CD86 molecules (18–20). However, the role of JNK in the maturation of human DCs has not been well studied because of a lack of JNK-specific inhibitors. Recently, Bennett *et al.* (21) have characterized SP600125 as a novel inhibitor of JNK catalytic activity. This compound inhibits JNK1, JNK2 and JNK3 with a high specificity ($IC_{50}$: 0.04–0.09 μM) and decreases the phosphorylation of c-Jun (21). Subsequently, this inhibitor has been studied in several different laboratories to characterize the role of JNK in several cellular responses (22,23). In this study, we have investigated the role of JNK pathway in LPS-induced maturation of human monocyte-derived DCs.

## Methods

### *Chemical and immunological reagents*

SP600125 (a JNK inhibitor), SB203580 (a p38 MAPK inhibitor) and PD98059 (an ERK inhibitor) were obtained from Calbiochem (Darmstadt, Germany). The inhibitors were dissolved in DMSO (Nacalai Tesque, Kyoto, Japan). The final concentration of DMSO never exceeded 0.5% and had no toxic effects. Ovalbumin (OVA) was purchased from Sigma (St Louis, MO). FITC-labeled anti-CD80 mAb (mouse IgM, clone BB1) was purchased from PharMingen (San Diego, CA). FITC-labeled CD54 mAb (mouse IgG1, clone 84H10), phycoerythrin (PE)-labeled CD86 mAb (mouse IgG2b, clone HA5.2B7) and PE-labeled CD83 mAb (mouse IgG2b, clone HB15A) were from Immunotech (Marseille, France) and ECD-labeled streptavidin was from Beckman Coulter (Tokyo, Japan). Biotin-conjugated HLA-DR mAb (mouse IgG2a, clone CR3/43) was from Becton-Dickinson (Mountain View, CA) and biotin-conjugated CD40 mAb (mouse IgG1, clone BE-1) was from Ancell (Bayport, MN). FITC- and PE-labeled mouse IgG1, FITC- and PE-labeled mouse IgG2b, FITC-labeled mouse IgM (Immunotech) and biotin-conjugated mouse IgG2b (Immunotech) were used as isotype-matched controls. Mouse polyclonal IgG was obtained from Sigma and FITC-labeled dextran (molecular mass 40 kDa) was purchased from Molecular Probes (Eugene, OR).

### *Culture of DCs from buffy coats*

Buffy coats were obtained from healthy volunteer donors according to institutional guidelines. Peripheral blood mononuclear cells (PBMCs) were prepared by density centrifugation using Ficoll-Paque (Amersham Biosciences, Sweden). PBMCs ($50 \times 10^6$ cells) were incubated in 10 ml of RPMI 1640 medium supplemented with 5% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all media and supplements were from Gibco-BRL, Grand Island, NY) and incubated for 1 h at 37°C. Nonadherent cells were removed by extensive washing and the remaining adherent cells were recovered by scraping. The mean purity of purified $CD14^+$ cells was >90%. Cells were subsequently cultured in

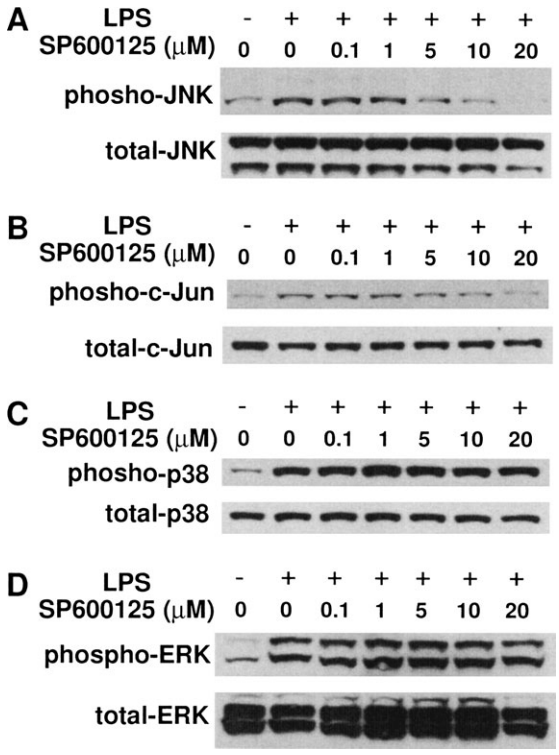



**Fig. 1.** SP600125 inhibition of LPS-induced JNK phosphorylation of DCs stimulated with LPS. Peripheral blood monocytes were differentiated into immature DCs after culturing with GM-CSF and IL-4. At day 5, immature DCs were pretreated with or without SP600125 (0.1–20 μM), SB203580 (50 μM) and PD98059 (40 μM) in the presence of 20 ng/ml LPS. The phosphorylation of JNK, c-Jun, p38 MAPK and ERK was detected by western blotting. Membranes were incubated with antibodies raised against the phosphorylated form of p46/54 JNK, c-Jun, p38 MAPK or p42/44 ERK. Membranes were then reprobed with antibodies against total p46/54 JNK, total c-Jun, total p38 MAPK or total p42/44 ERK for loading controls. Blots are representative of results obtained in three separate experiments.

6-well plates ($3 \times 10^6$ cells/well) in 3 ml of RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 10 mM HEPES, 1% nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 mM 2-ME (Sigma), 10 ng/ml GM-CSF (PeproTech, Rocky Hill, NJ) and 50 ng/ml IL-4 (PeproTech) for 5 days. Cells were fed with 1.5 ml of fresh medium containing 20 ng/ml of GM-CSF and 100 ng/ml of IL-4 on days 2 and 4. To obtain mature DCs, immature DCs ($4 \times 10^5$/ml) were then stimulated with 20 ng/ml LPS (*Escherichia coli*, strain 055:B5, Difco, Detroit, MI) in 1 ml of medium containing 10 ng/ml GM-CSF and 50 ng/ml IL-4. The MAPK inhibitors, SP600125, SB203580 and PD98059, were added 1 h before the LPS stimulation.

### *Flow cytometric analysis*

Cultured DCs were washed, resuspended at a concentration of 0.5–1 $\times 10^5$ cells in 50 μl of cold PBS containing 0.1% sodium azide, 10 mg/ml BSA and 200 μg/ml mouse IgG (Sigma) and incubated for 15 min on ice. Subsequent staining with either labeled mAb or appropriate isotypic controls was performed for 30 min on ice. Stained cells were washed,

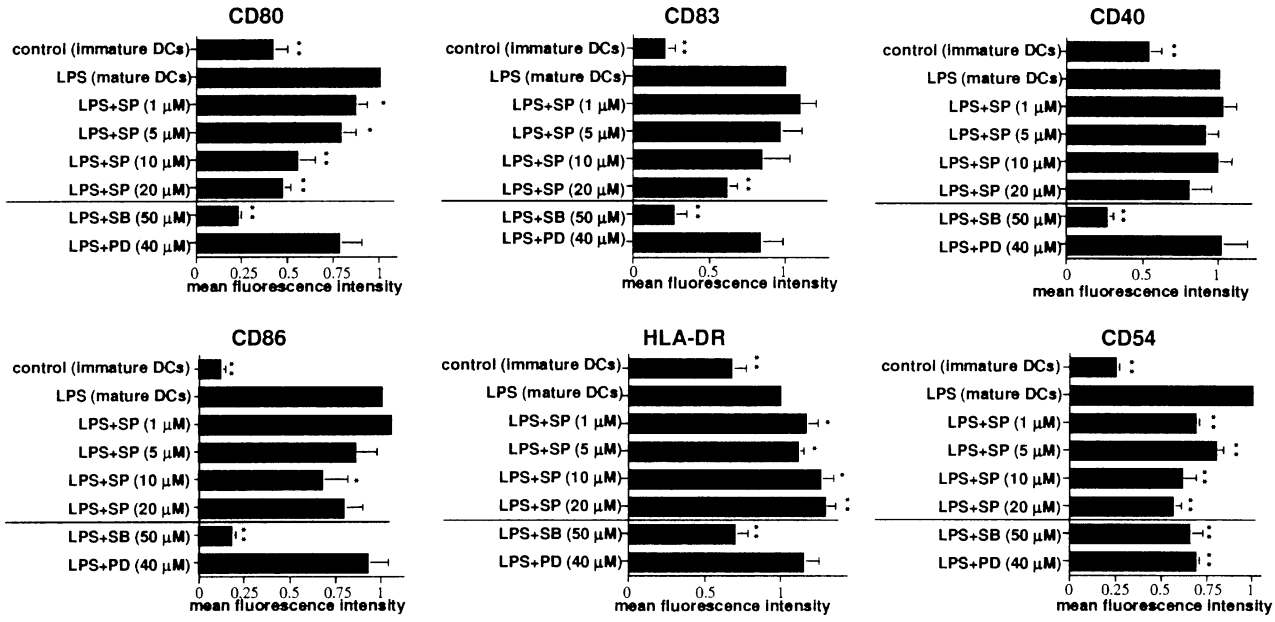



**Fig. 2.** Effects of SP600125, SB203580 and PD98059 on the phenotypic maturation of DCs after LPS stimulation. Peripheral blood monocytes were differentiated into immature DCs after treatment with GM-CSF and IL-4. At day 5, DCs were stimulated with 20 ng/ml LPS for 48 h in the presence or absence of SP600125 (1–20 μM), SB203580 (50 μM) or PD98059 (40 μM). The surface expression of CD86, CD80, CD83, CD54, CD40 and MHC class II molecules was analyzed by a flow cytometry. The results are shown as mean fluorescence intensity ± SEM. The mean fluorescence intensity of DCs treated with LPS alone was arbitrarily considered to be 1. $*P < 0.05$ and $**P < 0.01$ compared with LPS alone.

resuspended in 300 μl of cold PBS containing 0.1% sodium azide, 10 mg/ml BSA and 10 μg/ml 7-amino actinomycin D (Sigma) and analyzed for 3-color immunofluorescence by flow cytometry (Coulter, Tokyo, Japan). Cellular debris was eliminated from the analysis using a gate on forward and side scatter. A viability gate was set using 7-amino-actinomycin D, which allows discrimination between viable, necrotic and apoptotic cells (24). A minimum of $10^4$ cells was analyzed for each sample. Results were processed using Flow Jo software (Treestar, San Carlos, CA).

### *FITC-labeled dextran uptake*

Cells were incubated with FITC-labeled dextran (0.1 mg/ml) either at 4°C (internalization control) or at 37°C for 1 h. Cells were washed twice with a cold PBS containing 0.1% sodium azide and 10 mg/ml BSA and analyzed by flow cytometry.

### *Allogenic mixed lymphocyte reaction (MLR)*

Allogenic T cells were obtained from peripheral blood of healthy adults after Ficoll-Paque gradient centrifugation followed by adherence to plastic for 1 h at 37°C and passage over a nylon wool column (Wako, Osaka, Japan). The purity of $CD3^+$ T cells in the recovered cells was always >90%. $CD3^+$ T cells were distributed at a concentration of $1 \times 10^5$ cells per well into round-bottomed 96-well microplates and incubated for 5 days in the presence of graded numbers of irradiated (3000 rad, $^{137}Cs$ source) mature DCs that had been pretreated with SP600125, SB203580 or PD98059 in 200 μl of medium containing 10% FCS. T cell proliferation was assessed after 8–14 h incorporation of [$^3$H]thymidine (1 μCi/well; New England Nuclear, Boston, MA) by standard procedures. The results are expressed as the mean of triplicate cultures. The SEM of the results never exceeded 15%.

### *Production of TNF-α, IL-12 p40, IL-12 p70, IL-6 and IFN-β from DCs*

The amount of TNF-α, IL-12 p40, p70, IL-6 and IFN-β in each supernatant was measured using cytokine specific ELISA kits (BioSource and Endogen, Woburn, MA) according to the manufacturers' protocols.

### *Western blot analysis of cellular MAPKs*

After treatment with SP600125 (0.1–20 μM), SB203580 (50 μM) or PD98059 (40 μM) for 1 h, immature DCs were stimulated with LPS (20 ng/ml) for 30 min. Cells were washed twice with cold PBS and incubated with 60 μl of lysis buffer (50 mM Tris–HCl, 1% Triton X-100, 150 mM NaCl, 10% glycerol, 2 mM EDTA, 2 mM EGTA, 40 mM glycerophosphate, 50 mM NaF, 10 mM sodium pyrophosphate, 200 μM $Na_3VO_4$, 0.3 mM leupeptin, 1 mM pepstatin A, 1 mM PMSF and 100 nM okadaic acid, pH 7.4). The homogenates were centrifuged at 15 000 r.p.m. for 15 min at 4°C. Cell lysates were electrophoresed on 4–12% gradient Bis–Tris gels and transferred to nitrocellulose membranes for western blot analysis. After blocking with 5% fat-free dried milk for 1 h at room temperature, membranes were incubated for 2 h with antibodies raised against phosphorylated p42/44 ERK, p38 MAPK, p46/54 JNK, c-Jun or CREB (Cell Signaling, Beverly, MA). The membranes were washed and incubated with horseradish peroxidase-labeled goat anti-rabbit IgG (Bio-Rad, Hercules, CA) for 90 min. Immunoreactive bands were visualized by ECL detection reagent (Amersham, Piscataway, NJ). After stripping, the

membranes were reprobed with either anti-total p38 MAPK, p42/44 ERK, p46/54 JNK, c-Jun or CREB antibody (Cell Signaling) as loading controls.

*Measurement of T cell-derived IFN-γ and IL-4 after primary immunization*

Immature DCs were obtained from PBMCs after culturing with GM-CSF and IL-4. Purified autologous T cells ($10^6$/well) were co-cultured in a 24-well plate with OVA (100 μg/ml) pulsed immature DCs ($10^5$/well) or OVA (100 μg/ml) pulsed LPS-stimulated mature DCs pretreated with or without SP600125. After 5 days of co-culture, T cells were harvested and re-stimulated with OVA (10 μg/ml) pulsed autologous PBMCs in a 96-well plate. After 48 h, supernatants were collected and IFN-γ and IL-4 were assayed using ELISA kits (BioSource and Endogen) according to the manufacturers' instructions.

*Statistical analysis*

Data were analyzed using Student's *t*-test and a *P*-value $< 0.05$ was considered to be statistically significant.

## Results

*SP600125 specifically inhibits the phosphorylation of JNK, but not of p38 MAPK or ERK*

JNK, p38 MAPK and ERK are known to be phosphorylated during the maturation process of DCs induced by LPS (16). The phosphorylation of the MAPKs in immature DCs was weak, but LPS up-regulated the phosphorylation of JNK, as well as p38 MAPK and ERK, in DCs during maturation (Fig. 1). SP600125 inhibited the phosphorylation of JNK in a dose-dependent manner (Fig. 1A). SP600125 also inhibited the phosphorylation of c-Jun in a dose-dependent manner (Fig. 1B). In contrast, SP600125 did not affect the phosphorylation of p38 MAPK (Fig. 1C) and ERK (Fig. 1D). Doses higher than 20 μM of SP600125 were not used, as these concentrations were cytotoxic as determined by the trypan blue exclusion test. We also confirmed that SB203580 specifically inhibited the phosphorylation of p38 MAPK and PD98059 inhibited the phosphorylation of ERK (data not shown) (16–19).

*SP600125 inhibits the expression of CD54, CD80 and CD83, but augments the MHC class II expression of mature DCs*

In the LPS induced maturation process of DCs, the surface expression of CD40, CD54, CD80, CD83, CD86 and MHC class II molecules is up-regulated. The up-regulation of CD80, CD86, CD83 and CD54 induced by LPS was slightly inhibited by SP600125 (Fig. 2). Conversely, the expression of HLA-DR was augmented in a dose-dependent manner, while the expression of CD40 was not modified by SP600125. On the other hand, the expression of CD80, CD86, CD40, CD83 and HLA-DR induced by LPS was completely abrogated by SB203580, however, the expression of these surface molecules was not affected by PD98059 (Fig. 2) as has been described previously (16,17,19). The expression of CD54 was partially, but consistently, inhibited by SP600125, SB203580 and PD98059 (Fig. 2). These results indicated that JNK, p38 MAPK and ERK differentially regulate the phenotypic maturation of DCs after LPS stimulation.

*SP600125 only partially prevents the down-regulation of endocytosis in LPS-stimulated DCs*

Immature DCs exhibit high levels of endocytotic activity, but this function is down-regulated during the maturation process. The endocytotic function can be measured by the uptake of FITC-labeled dextran, which is mediated by the mannose receptor (4). The potent endocytosis of FITC-labeled dextran by immature DCs was profoundly down-regulated by LPS (Fig. 3A and B). SP600125 dose-dependently, but only slightly,

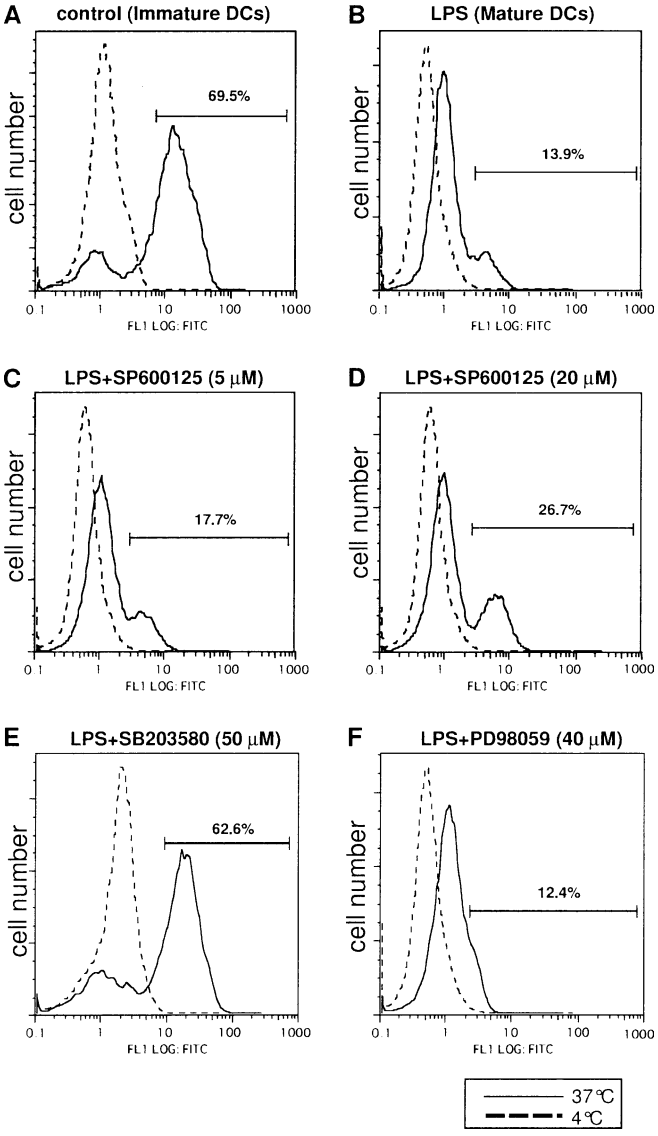
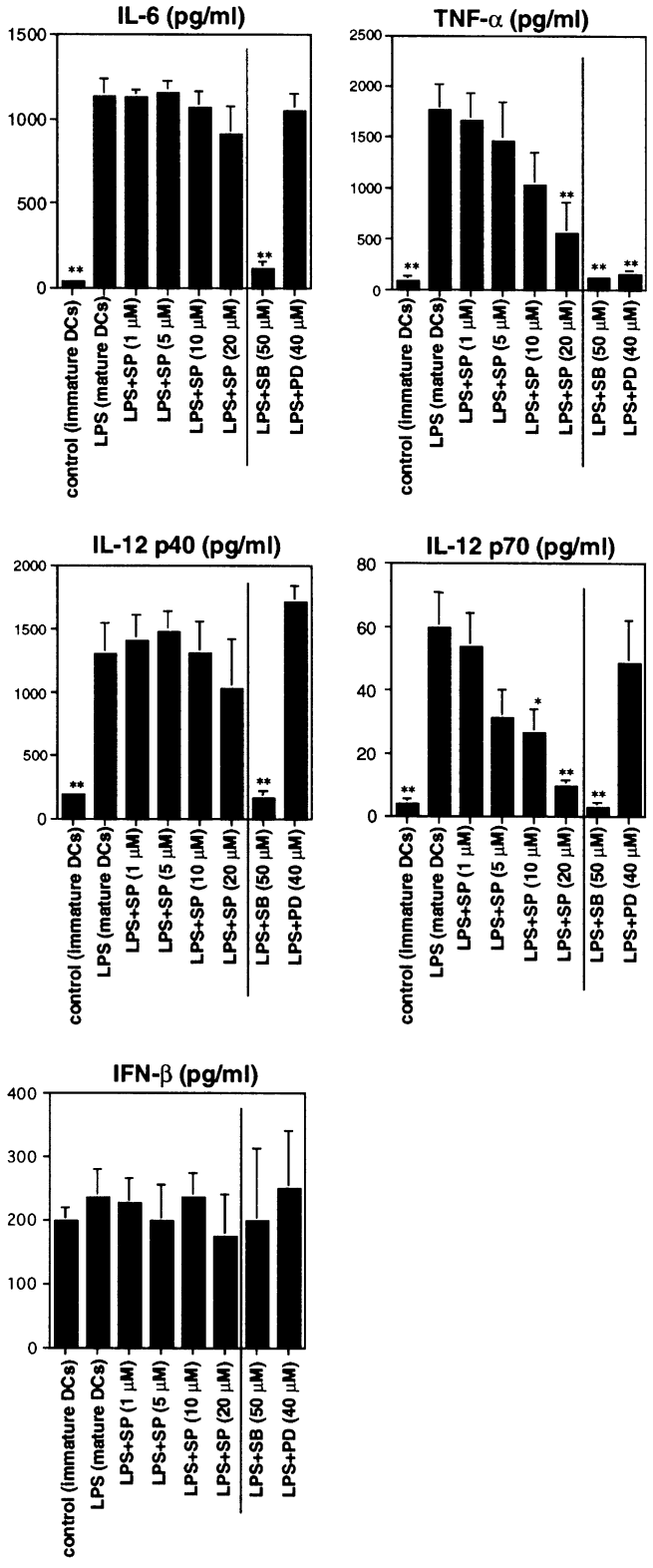



**Fig. 3.** Effects of SP600125 on the endocytosis of FITC-labeled dextran. Peripheral blood monocytes were differentiated into immature DCs after treatment with GM-CSF and IL-4. At day 5, DCs were stimulated with 20 ng/ml LPS for 48 h in the presence or absence of SP600125 (1–20 μM), SB203580 (50 μM) or PD98059 (40 μM). DCs were incubated with FITC-labeled dextran for 1 h at 4°C (thin lines) or 37°C (bold lines) and analyzed by a flow cytometry. These results are representative of three independent experiments.

inhibited the down-regulation of endocytosis induced by LPS (Fig. 3C and D). In contrast, SB203580 almost completely inhibited the LPS-induced down-regulation of endocytosis, while PD98059 had no effect (Fig. 3E and F). Thus, the functional maturation, as monitored by loss of endocytotic ability, was completely inhibited by the inhibitor of p38 MAPK, only partially by the JNK inhibitor, but not at all by the ERK inhibitor.




### *SP600125 inhibits the release of IL-12 p70 and TNF-α, but not of IL-6, IL-12 p40 and IFN-β from LPS-stimulated mature DCs*

DCs secrete many cytokines such as TNF-α, IL-6, IFN-β, IL-12 p40 and IL-12 p70 during LPS induced maturation. We studied the effects of SP600125, SB203580 or PD98059 on the secretion of these cytokines. Immature DCs produced very low amounts of TNF-α, IL-6, IL-12 p40 and IL-12 p70, and LPS significantly enhanced the production of these cytokines (Fig. 4). Although SP600125 inhibited the production of TNF-α and IL-12 p70 from mature DCs in a concentration-dependent manner, it did not affect IL-6, IFN-β and IL-12 p40 secretion (Fig. 4). In contrast, SB203580 almost completely abrogated the production of IL-6, IL-12 p40, IL-12 p70 and TNF-α. PD98059 inhibited the secretion of TNF-α, but not of IL-6, IL-12 p40 and IL-12 p70 (Fig. 4). Neither SB203580 nor PD98059 significantly affect the production of IFN-β. The production of these five cytokines was, therefore, differentially regulated by three MAPKs signaling pathways.

### *SP600125 does not inhibit the allostimulatory capacity of mature DCs*

Mature DCs exhibit more potent allostimulatory function than those of immature DCs (3). We tested whether SP600125 affects the allostimulatory function of mature DCs. As shown in Figure 5, allostimulatory function of immature DCs was up-regulated by LPS. It is of note that the allostimulatory capacity of mature DCs was slightly augmented in the presence of SP600125 (Fig. 5). PD98059 also slightly enhanced the allostimulatory function of mature DCs. In contrast, SB203580 significantly inhibited the allostimulatory function of mature DCs as previously described (11).

### *SP600125-pretreated DCs induce IL-4 production in OVA-specific T cell response*

Because the secretion of IL-12 p70 was impaired in mature DCs treated with SP600125, it was possible that SP600125-pretreated DCs might induce a Th2-prone immune response. To test this hypothesis, OVA-pulsed immature DCs or OVA-pulsed LPS-stimulated mature DCs pretreated with or without SP600125 were used as stimulator cells to prime autologous T cells. After co-culturing, the *in vitro* primed T cells were restimulated with autologous OVA-loaded PBMC and the secretion of IFN-γ and IL-4 was quantified. Upon restimulation with OVA, the OVA-pulsed mature DCs did prime the naive

**Fig. 4.** Effects of SP600125 on cytokine production by mature DCs. Peripheral blood monocytes were differentiated into immature DCs after treatment with GM-CSF and IL-4. At day 5, DCs were stimulated with 20 ng/ml LPS for 48 h in the presence or absence of SP600125 (1–20 μM), SB203580 (50 μM) or PD98059 (40 μM). The production of TNF-α, IL-6, IFN-β, IL-12 p40 and IL-12 p70 was measured by ELISA. Data represent mean ± SEM of four independent experiments. $*P < 0.05$ and $**P < 0.01$ compared with LPS stimulation alone.

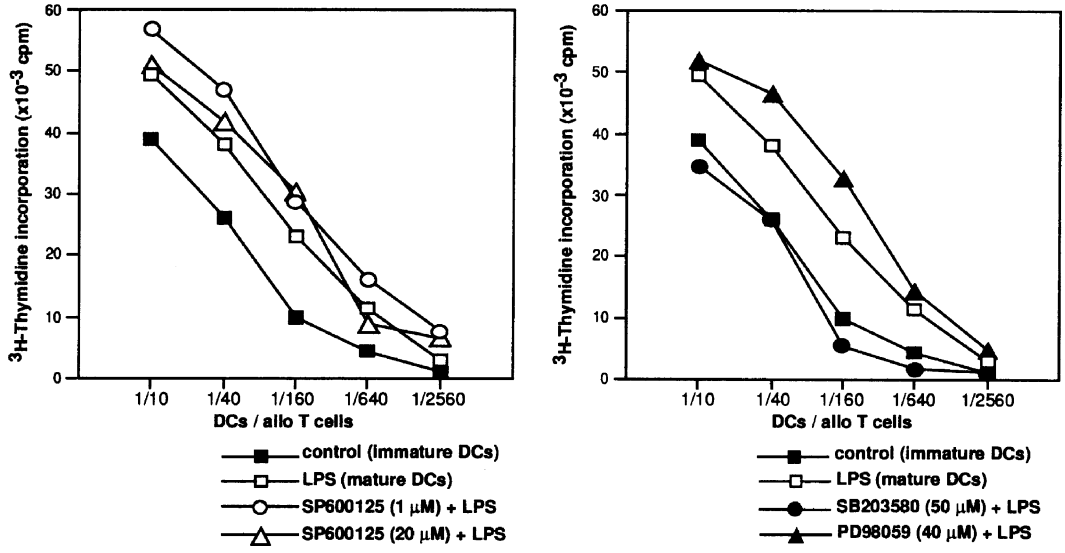



**Fig. 5.** Effects of SP600125 on the allostimulatory function of mature DCs. Peripheral blood monocytes were differentiated into immature DCs after treatment with GM-CSF and IL-4. At day 5, DCs were stimulated with or without 20 ng/ml LPS for 48 h in the presence or absence of SP600125 (1–20 μM), SB203580 (50 μM) or PD98059 (40 μM). Cells were further co-cultured with allogeneic T cells for 5 days in triplicate. The proliferation of T cells was measured by [$^3$H]thymidine incorporation assay. Data are shown as mean values. These results are representative of four independent experiments.

T cells in a Th1-prone fashion to produce IFN-γ, but not IL-4 (Fig. 6). In contrast, SP600125-pretreated mature DCs were capable of inducing the production of substantial amounts of IL-4 from T cells without significantly affecting the IFN-γ production (Fig. 6), suggesting that SP600125-pretreated DCs might skew the immune response toward a Th2 differentiation.

*SP600125 increases the phosphorylation of CREB*

Some agents, including histamine, cholera toxin, prostaglandin D2 and prostaglandin E2, inhibit IL-12 production in DCs, and shift the Th balance toward the Th2 phenotype (25–27). As these molecules induce a CREB phosphorylation (28), we next examined whether SP600125 might enhance the phosphorylation of CREB in DCs (Fig. 7). It was the case. The phosphorylation of CREB in immature DCs was weak, but LPS slightly up-regulated the phosphorylation of CREB in DCs during maturation (Fig. 7). SP600125 increased the phosphorylation of CREB in a dose-dependent manner (Fig. 7).

## Discussion

DCs are considered to be the most powerful antigen-presenting cells capable of priming resting T cells. The DC maturation process represents a crucial step in the initiation of an adaptive immune response (29). This maturation process is regulated by various extracellular stimuli including cytokines, bacterial products and membrane-bound ligands (4,30). The maturation of DCs is accompanied by changes in their morphological, phenotypic and functional properties (2). LPS is the major surface membrane component of gram-negative bacteria and can strongly stimulate the innate immune system. LPS is recognized by toll-like receptor 4 (TLR4), the signal is transmitted through pathways remarkably similar to IL-1 and IL-18 receptors, and ultimately leads to the activation of MAPKs (31–33).

Recent studies demonstrated that the blockade of the p38 MAPK pathway by SB203580 inhibited the up-regulation of CD40, CD80, CD86, CD83 and HLA-DR expression and profoundly reduced both the up-regulation of allostimulatory capacity and down-regulation of endocytotic activity of DCs induced by LPS (16,34). In contrast, specific inhibition of the ERK pathway by PD98059 did not inhibit, or only slightly augmented, the DC maturation (18,19).

The role of JNK in the maturation of DCs has not been well studied. SP600125 has recently been reported to be a specific JNK inhibitor (21). In our study, SP600125 inhibited the phosphorylation of JNK, but did not affect the phosphorylation of both p38 MAPK and ERK. The inhibition of JNK by SP600125 resulted in decreased expression of CD80, CD86, CD83 and CD54 and increased expression of MHC class II on LPS-stimulated DCs. The effects of SP600125 were different from those manifested by SB203580 and PD98059. However, the inhibitory action of SP600125 on the expression of CD80, CD86, CD83 and CD54 was weaker than that of SB203580. In addition, the reduced endocytosis during maturation was only slightly inhibited by SP600125, but completely inhibited by SB203580. In contrast to the inhibition of surface expression of the costimulatory and adhesion molecules (CD54, CD80 and CD86), SP600125 slightly augmented the allostimulatory capacity. The augmentation may be partly caused by the enhanced expression of MHC class II molecules by this compound. The inhibition of the Erk pathway by PD98059 also led to the up-regulation of allostimulatory function of DCs, but SB203580 profoundly down-regulated the allostimulatory function of DCs.

Unlike the immature DCs, LPS-stimulated mature DCs produce large amounts of proinflammatory and immunomodulatory cytokines (3,7,35). The findings that the LPS-stimulated mature DCs produced large amounts of IL-6, IL-12 p40, IL-12 p70 and TNF-α was confirmed in our study. In the present

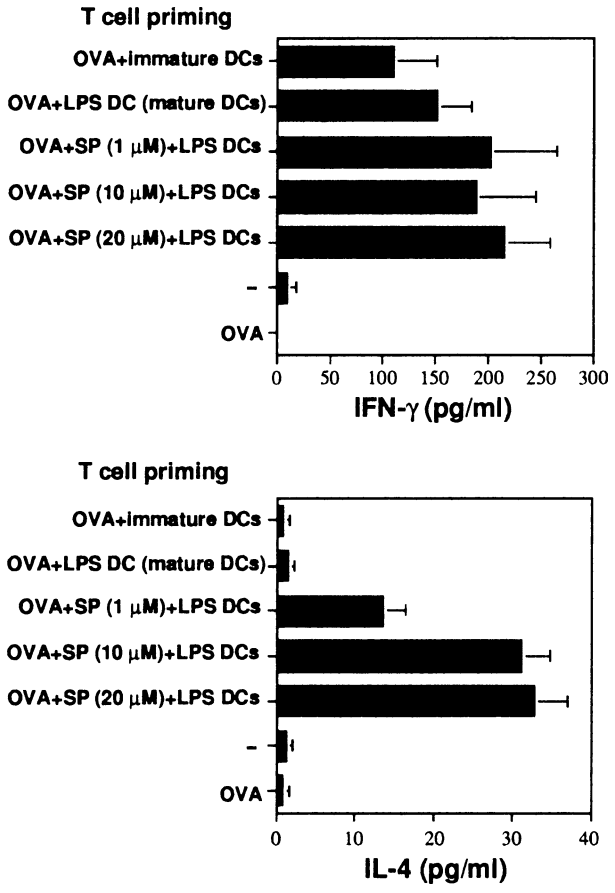



**Fig. 6.** SP600125-pretreated DCs induce IL-4 production in OVA-specific T cell response. T cells were primed with autologous OVA-pulsed immature DCs or autologous OVA-pulsed mature DCs pretreated with or without SP600125. After 5 days of co-culture, the primed T cells were harvested and then restimulated with autologous OVA-pulsed PBMC. After 48 h, supernatants were collected and the levels of IFN-γ and IL-4 were quantified. The data are shown as mean ± SEM and represent four independent experiments.

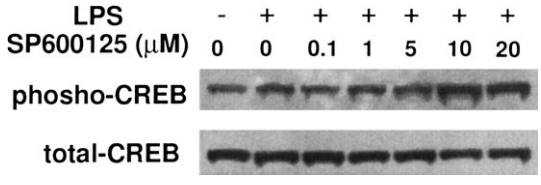



**Fig. 7.** SP600125 increase of LPS-induced CREB phosphorylation of DCs stimulated with LPS. Peripheral blood monocytes were differentiated into immature DCs after culturing with GM-CSF and IL-4. At day 5, immature DCs were pretreated with or without SP600125 (0.1–20 μM). The phosphorylation of CREB was detected by western blotting. Membranes were incubated with antibodies raised against the phosphorylated form of CREB. Membranes were then reprobed with antibodies against total CREB for loading controls. Blots are representative of results obtained in three separate experiments.

study, SB203580 strongly inhibited the production of all four cytokines, as has been demonstrated previously (16,34). SP600125 inhibited the secretion of IL-12 p70 and TNF-α in a dose-dependent manner, but not the production of IL-6 and IL-12 p40. Interestingly, PD98059 inhibited the production of TNF-α, but not the production of IL-6, IL-12 p40 or IL-12 p70. These phenotypic and functional data indicate (i) that p38 MAPK, JNK and ERK differentially regulate the maturation pathway of DCs, (ii) that p38 MAPK is essential to achieve the professional immunostimulatory function and (iii) that the incomplete inhibition of DC maturation by SP600125 or PD98059 is not sufficient to down-regulate the allostimulatory function of DCs.

JNK comprises JNK1, JNK2 and JNK3, and SP600125 inhibits all of the components (21,36). JNK1 and JNK2 are ubiquitously expressed in several tissues, whereas JNK3 is expressed more selectively in brain, testis and heart. The JNK3 gene has been shown to be involved in neuronal cell death (37), whereas JNK1 and JNK2 have been implicated in Th1/Th2 cell differentiation (38,39). Yang and coworkers demonstrated that the differentiation of precursor $CD4^+$ T cells into effector Th1, but not Th2, cells was impaired in JNK2-deficient mice because the JNK2-deficient murine $CD4^+$ T cells were unable to produce IFN-γ in response to IL-12 (38). JNK1-deficient murine T cells were also shown to preferentially differentiate into Th2 cells (39).

In addition to the direct effects of JNK on T cell differentiation, our study demonstrated that DCs were also the target cells for immunomodulation by JNK inhibitor. Human T cells primed by OVA-pulsed mature DCs produced only IFN-γ, but not IL-4. However, OVA-pulsed SP600125-treated mature DCs could initiate IL-4 production from T cells. SP600125 inhibited the production of the active form of IL-12 p70 from DCs without suppressing the allostimulatory function. It was of note that SP600125-treated DCs did not reduce the production of IFN-γ from T cells even though SP600125 inhibited the production of the active form of IL-12 p70 from DCs without suppressing the allostimulatory function. The reason is unclear at the moment but it might be due to the human/murine species difference.

A number of agents, including histamine, cholera toxin, prostaglandin D2 and prostaglandin E2 inhibit IL-12 production in DCs via the G protein–adenylyl cyclase pathway, and shift the Th balance of responding T cells toward the Th2 phenotype (25–27). These molecules have been shown to favor Th2 polarization by increasing intracellular cAMP in DCs. After increasing intracellular cAMP, they were able to induce the PKA activation and the transcription factor CREB phosphorylation (28). CREB binds to the coactivator CBP after phosphorylation, and CBP is shared by several transcription factors including c-jun (40). Coactivator competition has been shown to play an important role in the regulation of gene expression (41,42). It could be possible that inhibition of JNK-c-jun signaling would allow CBP to be available for CREB activation. Indeed, in our study, SP600125 increased the LPS-induced phosphorylation of CREB in DCs. Thus, one possible mechanism by which SP600125 causes Th2 polarization is associated with the phosphorylation of the transcription factor CREB like other Th2 inducers.

It is also possible that SP600125 tilts the T cell responses toward the Th2 phenotype by acting at the level of costimulatory molecules. CD86 is required for the development of Th2 responses (43,44). Our data indicated that SP600125 only slightly inhibits the expression of CD86 whereas strong inhibition was observed for the CD80 expression. Thus,

SP600125 might induce a Th2 response by decreasing the CD80/CD86 ratio in DCs.

In conclusion, the JNK signaling pathway has a role in the maturation process of DCs that is distinct from those of the p38 MAPK and ERK cascades and may be involved in the augmentation of the Th2-prone T cell response when it is suppressed.

## Abbreviations

| | |
|---|---|
| CBP | CREB-binding protein |
| CREB | cAMP response element binding protein |
| DCs | dendritic cells |
| ERK | extracellular signal-regulated kinase |
| GM-CSF | granulocyte macrophage-colony stimulating factor |
| JNK | c-Jun N-terminal kinase |
| MAPKs | mitogen-activated protein kinases |
| OVA | ovalbumin |
| PBMCs | peripheral blood mononuclear cells |
| PE | phycoerythrin |
| TLR | toll-like receptor |

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