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Original Article

Low-Dose Lipopolysaccharide Modifies the Production of IL-12 by Dendritic Cells in Response to Various Cytokines

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Dendritic cell (DC) activation is triggered by cytokines, including tumor necrosis factor (TNF)-α, and microbe components, including lipopolysaccharide (LPS). During the initial stage of infection, the microbe components appear to be present at low concentration. To determine the role of low-dose microbe-components in DC activation during the initial stage of infection, we examined the effects of low-dose LPS on cytokine-induced maturation and function of DCs. Low-dose LPS (1 ng/ml) treatment of DCs had only additive effects on the expression of CD86 and major histocompatibility complex class II induced by various cytokines, including interleukin (IL)-1β, TNF-α and interferon (IFN)-γ. IL-1β alone significantly induced IL-12 production in DCs, whereas TNF-α or IFN-γ induced modest levels of IL-12 production. When low-dose LPS (1 ng/ml), which only slightly induced IL-12 production, was added to the culture, only an additive effect was seen on IL-1β-induced IL-12 production. In contrast, low-dose LPS synergistically enhanced TNF-α- or IFN-γ-induced IL-12 production. SB203580, a specific inhibitor of p38 MAPK, markedly inhibited TNF-α- or IFN-γ-induced IL-12-production either in the absence or presence of LPS, but showed only modest effects on IL-1β-induced IL-12-production. These findings suggest that the p38 MAPK pathway is essential for the synergistic IL-12 production induced by TNF-α- or IFN-γ in combination with low-dose LPS in DC.
Key words lipopolysaccharide, IL-12, dendritic cells, TNF-α

INTRODUCTION

Dendritic cells (DC) are the most potent antigen presenting cells (APC) and play a major role in the initiation and regulation of the adaptive immune response to antigens[1-3]. Upon encountering foreign antigens, DCs are rapidly activated by a complex process and become mature. DC activation in sites of inflammatory response is triggered by cytokines, including tumor necrosis factor (TNF)-α, and bacterial components, such as lipopolysaccharide (LPS)[1,2,4]. Mature DCs have high surface expression of major histocompatibility complex (MHC) and costimulatory molecules including CD80, CD86, and CD40. The mature DCs migrate from peripheral tissues to the T cell area of draining lymph nodes, where the DCs activate antigen-specific helper T cells (Th)[1,2]. Interleukin (IL)-12 produced by DCs during antigen presentation promotes T helper type 1 (Th1) differentiation, which results in enhancement of the cell-mediated immune response against various pathogens[5,6].

Certain microbial components activate DCs and macrophages via Toll-like receptors (TLR)[7,8]. The TLR-mediated activation of DCs is crucial to the initiation of acquired immune responses. However, during the initial stage of infection as well as chronic infection, the concentration of microbial components seems to be low. Thus, it is important to elucidate the influence of low-dose stimulants on DC function and the subsequent acquired immune response.

Winzler *et al*.[9] established a growth factor-dependent immature DC line from splenocytes of adult C57BL/6 mice. Using a similar DC *in vitro* differentiation system, a number of important findings have been reported and verified[10-15]. Following the method of Winzler *et al*., we have established a homogeneous immature DC line (BC1) from BALB/c splenocytes[16]. The aim of this study was to determine the role of low-dose microbial components in DC activation during the initial stage of infection. Using these BC1 cells, we examined the effects of low doses of LPS on cytokine-induced IL-12 production by DCs.

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MATERIALS AND METHODS

Reagents and antibodies (Ab)

Recombinant murine granulocyte-macrophage colony-

stimulating factor (GM-CSF), TNF-α, interferon (IFN)-γ, IL-1β, IL-4, and IL-6 were purchased from PeproTech (London, UK). LPS from *Escherichia coli* O55 : B5 was purchased from Sigma Chemical (St Louis, MO). SB203580 was obtained from Calbiochem (La Jolla, CA). Fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD86 monoclonal Ab (mAb) (GL1), biotin-conjugated anti-mouse I-A^d mAb (AMS-32.1), and streptavidin Cy-Chrome™ were obtained from PharMingen (La Jolla, CA). As control IgG, FITC-conjugated rat IgG2a and biotin-conjugated mouse IgG2b were purchased from PharMingen and Immunotech (Marseille, France), respectively.

Culture Media

Iscove's modified Dulbecco's medium (IMDM) (Sigma Chem. Co., St. Louis, MO) supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 IU/ml penicillin, 100 μg/ml streptomycin, 600 μg/ml L-glutamine, and 50 μM 2-mercaptoethanol (complete IMDM) was used for cell culture[16]. Fibroblast supernatants (SN) from NIH/3T3 cells were collected from confluent cultures grown in complete IMDM.

DC line (BC1)

The spleen-derived dendritic cell line (BC1) was generated from BALB/c mice as described in previous reports[9, 16]. BC1 cells were expanded in complete IMDM containing 30% NIH/3T3 SN and 10 ng/ml mouse recombinant GM-CSF (henceforth referred as R1 medium).

Flow cytometry

BC1 cells were incubated with culture SN of 2.4G2 hybridomas (rat anti-mouse FcγRII/III, CD32) to prevent Ab binding to FcRII/III, and then stained using FITC- or biotin-conjugated mAb and streptavidin-Cy-Chrome™. Flow cytometry was performed on EPICS® XL (Coulter Co. Miami, FL), as previously described[16, 17].

Stimulation of BC1 cells

Cells (2×10^4 cells) were cultured with LPS, IL-1β, TNF-α, IFN-γ, IL-4, or IL-6 in 1 ml of R1 medium in a 24-well culture plate at 37°C for 24 hr. In some experiments, cells were pretreated with SB203580 (10 μM) for 1 hr and then stimulated with low-dose LPS plus IL-1β, TNF-α or IFN-γ.

Measurement of IL-12 p40 by enzyme-linked immune adsorbent assay (ELISA)

After the culture of BC1 cells for 24 hr, culture SN were subjected to quantification of IL-12 p40 protein levels by ELISA using the OptEIA™ Set : Mouse IL-12p40 (PharMingen, San Diego, USA) and SUMIRON ML-1120T (TAUNZU, Numazu, Japan).

RESULTS

Effects of LPS, IL-1β, TNF-α, and IFN-γ on cell surface expression of CD86 and MHC class II and on IL-12 production in BC1 cells

We have reported that unstimulated BC1 cells are phenotypically and functionally immature DCs[16-19]. The immature BC1 cells were treated with LPS or various cytokines for 24 hr, and the cell surface expression of CD86 and MHC class II and the IL-12 production were evaluated by flow cytometry or ELISA, respectively (Fig. 1). LPS markedly increased the expression of CD86 and I-A^d on BC1 cells in a dose dependent manner (Fig. 1A). IL-1β and TNF-α also increased CD86 and I-A^d expression. In contrast, IFN-γ showed no significant effects on the expression of these proteins. Although TNF-α and LPS increased I-A^d expression to a similar extent, the effect of TNF-α on CD86 expression was moderate in comparison to that of LPS. The effect of IL-1β on CD86 and I-A^d expression was low compared to that of LPS.

On the other hand, both LPS and IL-1β significantly induced IL-12 production by BC1 cells in a dose dependent manner (Fig. 1B). The level of IL-12 in the LPS-treated culture was much higher than the level in the IL-1β-treated culture. In contrast, TNF-α or IFN-γ only slightly induced IL-12 production by BC1 cells at any concentrations examined.

From these results, we defined 1 ng/ml LPS as low-dose LPS ; this concentration showed slight but significant effects on DC phenotype and IL-12 production. We used this dose of LPS for the subsequent studies. In addition, all cytokines were used at 40 ng/ml, based on their optimal effects on DC phenotype and IL-12 production (Fig. 1).

Influence of low-dose LPS on IL-1β, TNF-α, or IFN-γ-induced expression of cell surface markers and IL-12 production by BC1 cells

We next examined the influence of low-dose LPS (1 ng/ml) on cytokine-induced expression of cell surface markers and IL-12 production in BC1 cells. As was shown in Fig. 1A, this dose of LPS alone slightly but significantly increased the expression of I-A^d and CD86 (Fig. 2A).

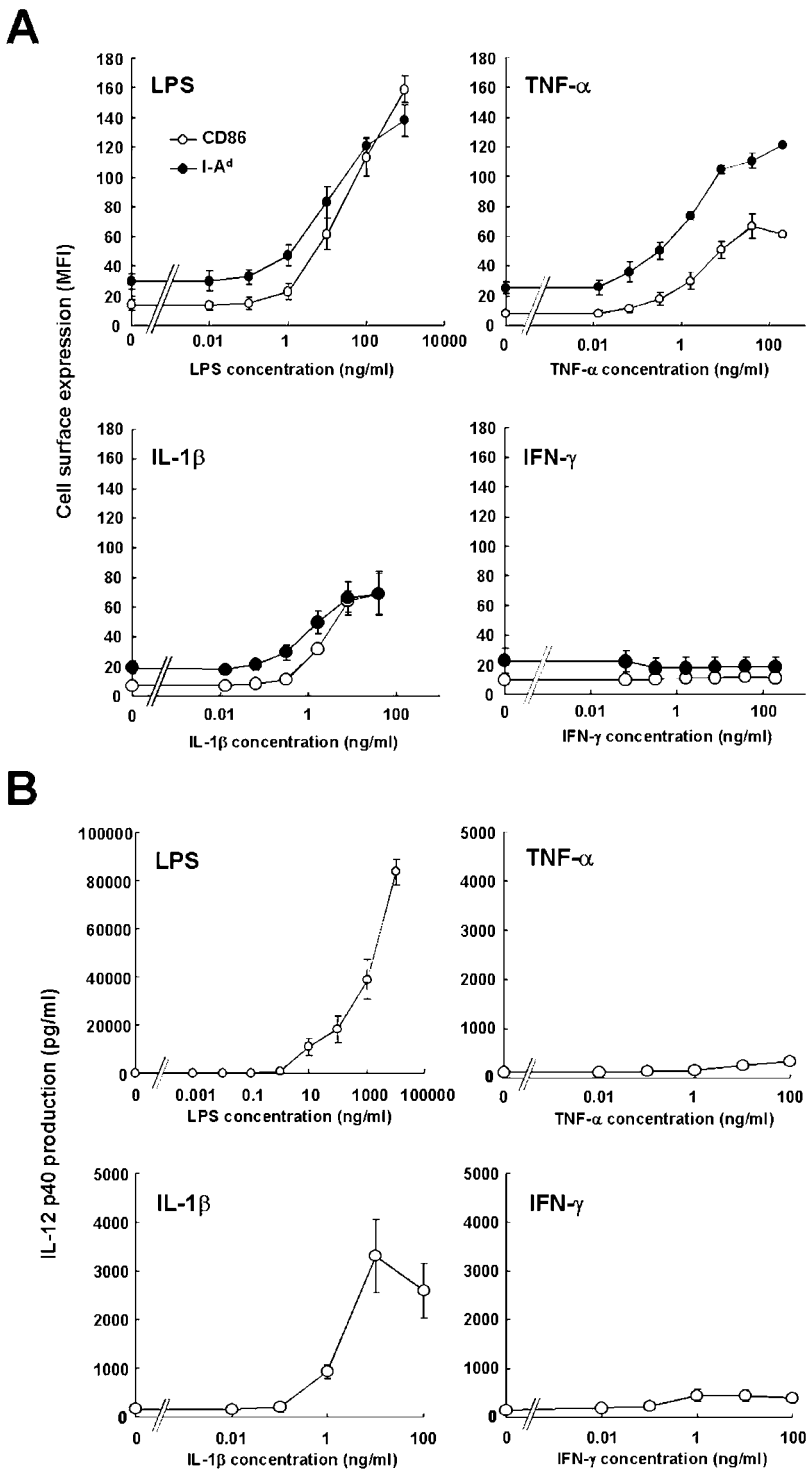

Fig. 1. Effects of LPS and various cytokines on the surface expression of CD86 and MHC class II and on IL-12 production by BC1 cells. BC1 cells were treated with LPS, IL-1β, TNF-α, or IFN-γ for 24 hr. Expression of CD86 and MHC class II on BC1 cells was analyzed by flow cytometry (A). The culture SNs were collected and the amounts of IL-12 were quantitated by ELISA (B). Each symbol represents the mean (mean ± SE) of three independent experiments.

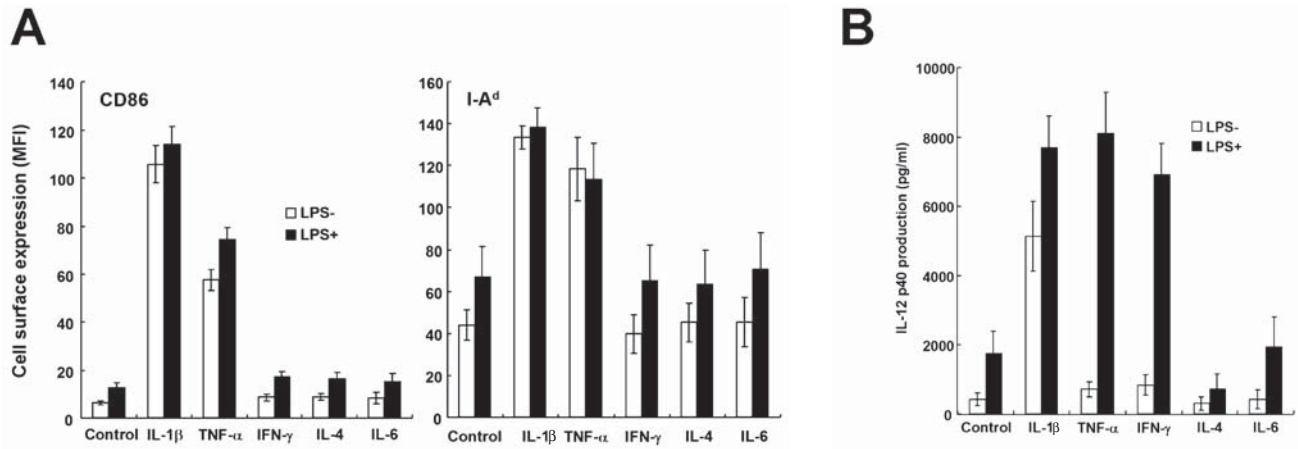

Fig. 2. Effects of low-dose LPS on cytokine-induced IL-12 production and expression of CD86 and MHC class II on BC1 cells. BC1 cells were treated with IL-1β, TNF-α, IFN-γ, IL-4, or IL-6 (40 ng/ml) in the presence or absence of low-dose LPS (1 ng/ml) for 24 hr. Expression of CD86 and MHC class II on BC1 cells was analyzed by flow cytometry (A). The culture SNs were collected and the amounts of IL-12 were quantitated by ELISA (B). Each column represents the mean (mean ± SE) of seven independent experiments.

Although IL-1β and TNF-α again significantly increased the expression of CD86 and I-A^d, neither IFN-γ, IL-4, nor IL-6 had a significant effect on expression. When low-dose LPS was added to cultures of BC1 cells stimulated with various cytokines, the cell surface expression of both CD86 and I-A^d increased slightly, except in the case of I-A^d expression induced by TNF-α. However, the influence of low-dose LPS on the cytokine-induced upregulation of CD86 and I-A^d appeared to be additive (Fig. 2A).

We then analyzed the influence of low-dose (1 ng/ml) LPS on IL-12 production by BC1 cells induced by various cytokines, as described above. Low-dose LPS alone induced slight but significant IL-12 production by BC1 cells (Fig. 2B). When low-dose LPS was added to cultures of BC1 cells stimulated with cytokines, marked enhancement of IL-12 production was observed in cells stimulated with either TNF-α or IFN-γ (Fig. 2B). This enhancement was regarded as synergistic. In contrast, low-dose LPS showed only an additive effect on IL-1β-induced IL-12 production. The moderate level of IL-12 production induced by low-dose LPS was decreased in the presence of IL-4 and was unaffected by IL-6 treatment (Fig. 2B).

Effects of SB203580, a specific inhibitor of the p38 MAPK pathway, on cytokine-induced IL-12 production by BC1 cells.

It has been reported that p38 MAPK is involved in IL-12 production by DCs and macrophages. We next examined the effects of SB203580, a specific inhibitor of the p38 MAPK pathway, on IL-12 production by BC1 cells that were stimulated with TNF-α or IFN-γ plus low-dose LPS (Fig. 3). BC1 cells were pretreated with SB203580 (20 μM) for 1 hr. Then, the cells were cultured with IL-1β, TNF-α, or IFN-γ plus low-dose LPS in the presence of the inhibitor. In either the presence or absence of low-dose LPS, SB203580 moderately inhibited the IL-1β-induced IL-12 production by BC1 cells (70% of the control level, IL-12 production by SB203580-untreated cells), and markedly inhibited TNF-α or IFN-γ-induced IL-12 production (less than 20% or 5% of the control, respectively). Thus, the synergistic enhancement of IL-12 production by treatment of BC1 cells with TNF-α or IFN-γ plus low-dose LPS appeared to be mediated mainly by the p38 MAPK pathway.

DISCUSSION

The activation and maturation of DCs is regulated by various extracellular stimuli, including cytokines, costimulatory molecules, and microbial components. These events are accompanied by alterations of the morphological, phenotypic, and functional properties of DCs. During the initial stage of infection as well as chronic infection, microbial components seem to be present at low concentrations. Thus far, few reports have examined the influence of low-dose microbial components on immune responses, including cytokine production by DCs. In this study, we analyzed the influence of low-dose LPS, a typical microbial component, on DC activation induced by various cytokines.

Low-dose (1 ng/ml) LPS was used in these experiments because this dose showed a minimal effect on IL-12 production by DCs compared with higher doses (i.e. 100 ng/ml) and this model might provide a good model of the initial stage of infection. When low-dose LPS was added to DCs (BC1 cells)

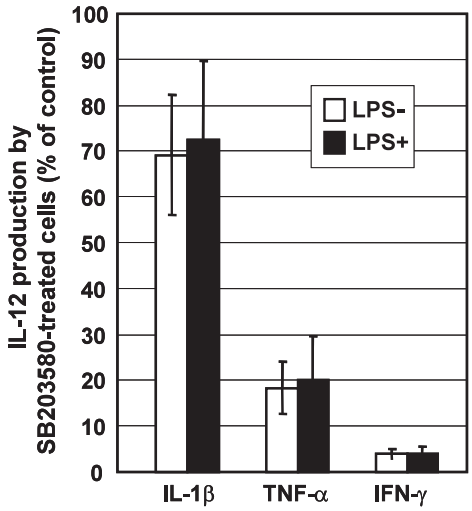

Fig. 3. Effects of a p38 MAPK inhibitor, SB203580, on cytokine-induced IL-12 production by BC1 cells in the presence or absence of low-dose LPS. BC1 cells were pretreated with SB203580 (20 μM) for 1 hr, then treated with IL-1β, TNF-α, or IFN-γ (40 ng/ml) plus low-dose LPS (1ng/ml) for 24 hr in the presence of the inhibitor. The culture SNs were collected and the amounts of IL-12 were quantitated by ELISA. The influence of SB203580 treatment on IL-12 production is shown as percent of control (IL-12 production by SB203580-treated cells/IL-12 production by SB203580-untreated cells). Each column represents the mean ± SE of three independent experiments.

stimulated with various cytokines, different effects were observed. Low-dose LPS showed only an additive effect on IL-1β-induced IL-12 production by DCs. In contrast, the same low dose of LPS synergistically enhanced TNF-α or IFN-γ-induced IL-12 production by DCs. Since TNF-α or IFN-γ alone induced IL-12 production by DCs only slightly in comparison to IL-1β, it seems that low doses of LPS may play a considerable role in DC activation by TNF-α or IFN-γ during the initial stage of infection. In contrast, low-dose LPS showed only an additive effect on the expression of CD86 and MHC class II induced by TNF-α or IFN-γ. These results suggest that the surface expression of MHC class II and CD86 and IL-12 production by DCs are regulated independently in the presence of TNF-α or IFN-γ and low-dose LPS.

MAPK appears to be involved in regulation of DC maturation and/or cytokine production by DCs[16, 20-25]. It has been reported that activation of p38 MAPK results in the induction of IL-12 production by DCs. In this study, SB203580 (a p38 MAPK inhibitor) markedly decreased TNF-α or IFN-γ-induced IL-12 production by DCs. In contrast, SB203580 showed only modest inhibitory effects on IL-1β-induced IL-12 production. These findings suggest that the p38 MAPK pathway is crucial for the IL-12 production induced by TNF-α or IFN-γ, but is not essential for IL-1β-mediated IL-12 production by DCs. The IL-1β-mediated IL-12 production may be predominantly mediated via other pathways, such as the NF-κB pathway. SB203580 also markedly inhibited TNF-α or IFN-γ-induced IL-12 production by DCs that was synergistically enhanced by the presence of a low-dose LPS. Thus, it seems that the p38 MAPK pathway is essential for IL-12 production induced by TNF-α or IFN-γirrespective of the presence or absence o low-dose LPS.

In this study, production of IL-12 p40 by DCs was evaluated. We also attempted to evaluate the production of IL-12 p70, the bioactive form of this cytokine ; however, IL-12 p70 was not detectable under our culture conditions (data not shown).

We have shown herein that TNF-α or IFN-γ-induced IL-12 production was selectively and considerably augmented by low-dose LPS. Only a slight effect of low-dose LPS was observed on the expression of I-A^d and CD86 induced by these cytokines. Since IL-12 production by DCs during antigen presentation promotes Th1 differentiation, it is important to elucidate the precise mechanism of IL-12 production by DCs in low-dose endotoxin environments ; this may lead to the development of the new regulation system of infectious diseases where Th1 type immune responses are effective to protect against the infectious agents.

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