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FEMS Immunology and Medical Microbiology 21 (1998) 303–311

FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY

Role of CD86 (B7-2) in triggering of antigen-specific IgE antibody response by lipopolysaccharide

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Received 27 April 1998; revised 8 June 1998; accepted 16 June 1998

Abstract

The role of CD86 in triggering of ascaris extract-specific IgE antibody response by lipopolysaccharide was studied. The simultaneous administration of anti-CD86 antibody with ascaris extract and lipopolysaccharide prevented the production of IgE antibody response to ascaris extract. $CD86^+$ cells were detected in peritoneal cavities and spleens of mice injected intraperitoneally with lipopolysaccharide. $CD86^+$ cells appeared in peritoneal cavities and spleens eight hours after lipopolysaccharide injection, and they were detectable for a week. $CD86^+$ cells in peritoneal cavities and spleens were mainly surface Ig-positive B-cells and some Ig-negative cells. It was suggested that lipopolysaccharide induced the expression of CD86 mainly on B-cells, and that $CD86^+$ cells induced by lipopolysaccharide injection might play an important role as antigen-presenting cells on triggering of ascaris extract-specific IgE antibody response by lipopolysaccharide.

Keywords: Lipopolysaccharide; CD86; IgE antibody response; Co-stimulatory molecule

1. Introduction

Co-stimulatory molecules play an important role in the regulation of immune response, especially in regulating the initial response of a T-cell to antigen [1–3]. The interaction between co-stimulatory molecules and their ligands allows peripheral T-cells to respond to activated B-cells by dividing and producing cytokines required for T-cell differentiation [1]. Two distinct co-stimulatory molecules, CD80 and CD86, have been identified [4–7]. CD80 and CD86 are known to be induced on the surface of antigen-presenting cells: B-cells, macrophages and dendritic cells [6,8,9], although they were originally defined as a B-cell activation marker [4–6]. Recently, it has been reported that CD80 and CD86 can have distinct effects on in vivo immune responses. In vivo, CD80 and CD86 molecules appear to differ in their ability to potentiate the differentiation of T-helper cells into either type 1 cells, which direct cell mediated immunity, or type 2 cells, which support a humoral immune response [3]. However, the exact role of CD80 and CD86 in regulation of in vivo immune response is still unclear.

Bacterial lipopolysaccharide (LPS) exhibits a vari-

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0928-8244/98/$19.00
PII: S0928-8244(98)00085-6

ety of immunological activities [10,11]. LPS can potentiate immune responses through activating antigen-specific helper T-cells [12–14]. It is likely that the activation of antigen-specific helper T-cells by LPS may require the expression of co-stimulatory molecules, such as CD86 and CD80, on antigen-presenting cells. Recently, the stimulation with LPS has been reported to influence CD86 and CD80 expression on antigen-presenting cells. CD86 and CD80 antigen is hardly detected on normal spleen cells and peritoneal cells, but the in vitro cultivation with LPS upregulates their expression on B-cells and macrophages [8,9,15–17]. Whereas LPS did not seem to upregulate CD86 levels in vitro on dendritic cells expressing low level of CD86 [8], LPS injection transiently upregulates in vivo expression of CD86 and CD80 on splenic dendritic cells [18]. However, there are few reports concerning in vivo expression of CD80 and CD86 on other cell types by administration of LPS. Moreover, the relationship between the expression of CD80 and CD86 and immunological activities of LPS is still unknown. It was supposed that co-stimulatory molecules might participate in triggering of helper T-cells in an adjuvant action of LPS on antibody response. In the present study, we investigated if and how co-stimulatory molecules participated in the potentiation of ascaris extract-specific antibody response by LPS. Here we demonstrate the upregulation of CD86 expression on peritoneal and spleen cells by administration of LPS and its participation in triggering of ascaris extract-specific IgE antibody response.

2. Materials and methods

2.1. Mice

BALB/c and SMA mice of approximately 6–8 weeks of age were used. SMA mice were bred at the Inbred Animal Breeding Laboratory, Aichi Medical University and BALB/c mice were purchased from Japan SLC (Hamamatsu, Japan).

2.2. Antibody

Rat monoclonal antibody (mAb) to mouse CD80 and CD86, and hamster mAb to mouse CD28 and CTLA-4 were purchased from Pharmingen, San Diego, CA, USA. Fluorescein isothiocyanate (FITC)-conjugated goat antibodies specific to rat IgG and hamster IgG were purchased from Cappel, West Chester, Pennsylvania, USA. Phycoerythrin (PE)-conjugated $F(ab')_2$ goat anti-mouse Ig antibody was purchased from Dako, Glostrup, Denmark.

2.3. LPS and administration

LPS from *Escherichia coli* O55, *E. coli* O111, and *Salmonella enteritidis* were obtained from Difco, Detroit, MI, USA. *Klebsiella* O3 LPS was extracted from *Klebsiella pneumoniae* LEN-1 (O3:K1−) by the phenol water method and purified by ultracentrifugation [19,20]. Rough-type LPS was prepared from *K. pneumoniae* LEN-113 (O3−;K1−), a mutant derived from strain LEN-1 [21]. Separation of the free lipid A and the O-specific polysaccharide fractions from *Klebsiella* O3 LPS was performed by heating at 100°C for 1 h in 1% acetic acid, and the polysaccharide fraction was further purified chromatography as described previously [22]. Various preparations of LPS were injected i.p. into mice. Three to four mice per experimental group were used for experiments.

2.4. Preparation of cell suspensions

Peritoneal cells were obtained by washing out the peritoneal cavity with 0.01 M phosphate-buffered saline (PBS) at pH 7.2. Lymphoid organs including the spleen, thymus and lymph node were dissected to prepare single-cell suspensions.

2.5. Immunofluorescent staining and flow cytometry

An aliquot of $5–10\times10^5$ fresh cells was suspended in a 1:20 dilution of rat anti-CD86 antibody, and incubated for 30 min on ice. The cells were washed three times with PBS containing 1% bovine serum albumin, and incubated in a 1:40 dilution of FITC-conjugated goat anti-rat IgG antibody (60 μl) for 30 min on ice. The cells were analyzed with a laser flow cytometry (FACS 440, Becton Dickinson, Palo Alto, USA). For two-color flow cytometric analysis, cells stained with anti-CD86 mAb were fur-

ther incubated with a 1:100 dilution of PE-conjugated goat anti-mouse Ig-specific antibody.

2.6. Immunohistochemical staining

Spleens from mice injected i.p. with LPS 16 h before was fixed in 10% formaldehyde. Paraffin sections of the spleen were deparafinized, and blocked the endogenous peroxidase activity with methanol containing 0.3% hydrogen peroxide for 15 min at room temperature. The sections were washed in PBS containing 2% normal horse serum and incubated overnight at 4°C with anti-CD86 mAb. Immune complexes were detected with avidin–biotin peroxidase complex (Vector, Burlingame, USA). Sections were counterstained with Methyl green. In negative control sections, an irrelevant antibody with the same Ig class was used.

2.7. Adjuvant activity of LPS on IgE antibody response to ascaris extract

Ascaris suum extract was purchased from LSL, Tokyo, Japan, and suspended at a concentration of 1 mg/ml in PBS. In preliminary experiments, the triggering of IgE antibody response to ascaris extract required the injected amount of more than 10 μg of LPS. There was no significant difference in triggering of IgE antibody response among LPS from *E. coli* O111, O55 or *S. enteritidis*. Based on these findings, the mixture of ascaris extract (200 μg) and *E. coli* O111 LPS (100 μg) with or without anti-CD86 or CD80 antibody (50 μg) was injected i.p. into mice, and blood was taken from the retro-orbital venous plexus 2 weeks after the injection. The amount of IgE antibody to ascaris extract in sera was determined by an enzyme-linked immunosorbent assay (ELISA) [23]. In brief, microplates were coated at room temperature overnight with ascaris extract (5 μg/ml). The plates were washed three times with PBS, and then blocked with 1% skim milk in PBS. The plates were washed with washing buffer (PBS with 0.05% Tween-20). Each serum sample was made by the two-fold dilution from 1/10 to 1/1280 with PBS and dispensed down a column of a microplate at 100 μl/well. The plates were washed, and treated with a 1:1000 dilution of rat anti-mouse IgE mAb (Experimental Immunology Unit, Brussels, Belgium) for 60 min. After treatment with horseradish peroxidase-conjugated rabbit anti-rat Ig antibody for a further 60 min, the peroxidase substrate solution (200 μl) containing 0.01% o-phenylene diamine and 0.03% H_2O_2 in PBS was added. Reactions were stopped about 15 min later by 1 N H_2SO_4. The optical density (OD) unit of wells was read relative to a substrate blank at 492 nm on an automatic microplate reader 450 (Bio-Rad, Herculus, CA, USA). The mean OD unit with standard deviation at various dilutions of sera in each experimental group (four mice) was calculated.

3. Results

3.1. Inhibitory effect of anti-CD86 mAb in IgE antibody production to ascaris extract by LPS

We studied the participation of CD86 in triggering of IgE antibody response to ascaris extract by LPS. Mice were immunized i.p. with the mixture of ascaris extract and LPS with or without anti-CD86 mAb, and 2 weeks later, the blood was taken from those immunized mice. The titer of IgE antibody to ascaris extract was estimated by ELISA. The experimental result is shown in Table 1. Immunization of mice with the mixture of ascaris extract and LPS caused a significant IgE antibody response to ascaris extract. However, simultaneous administration of anti-CD86 antibody prevented ascaris-specific IgE antibody response. The IgE antibody titer in sera from mice injected together with anti-CD86 antibody was approximately one-fifth as high as that in sera from

Table 1
Prevention of IgE antibody production to ascaris extract by anti-CD86 mAb

Materials injected	IgE antibody ($OD_{490} \pm$ S.D.)
None	0.01
Ascaris extract	0.02
Ascaris extract+LPS	0.734 ± 0.11
Ascaris extract+LPS+anti-CD86 mAb	0.128 ± 0.01
Ascaris extract+LPS+anti-CD80 mAb	0.698 ± 0.22

Mice were immunized i.p. with ascaris extract and LPS with or without anti-CD86 or CD80 antibody, and 2 weeks later, the blood was taken from those immunized mice. The titer of IgE antibody to ascaris extract was estimated by ELISA.

mice injected without the antibody. The administration of anti-CD86 antibody 48 h after the injection of ascaris extract and LPS failed to prevent IgE antibody response. The injection of ascaris extract alone or LPS alone did not result in significant IgE antibody production to ascaris extract. Anti-CD86 mAb did not prevented IgM and IgG antibody response to bovine serum albumin or keyhole limpet hemocyanin (data not shown). The administration of anti-CD80 mAb in place of anti-CD86 antibody did not prevent IgE antibody response to ascaris extract.

3.2. *Expression of CD86 on cells from various lymphoid organs of mice injected i.p. with LPS*

In the preceding paragraph, it was suggested that CD86 expression might be related to the adjuvant action of LPS on IgE antibody response. Therefore, we studied in vivo expression of CD86 by administration of LPS in order to elucidate the role of CD86 in triggering IgE antibody response by LPS. Mice were injected i.p. with LPS (100 μg), and 16 h after the injection the expression of CD86 on cells from various lymphoid organs, such as the spleen, thymus, lymph node, and peritoneal cavity was examined with the laser flow cytometry. Typical histograms of $CD86^+$ cells in peritoneal cells and spleen cells are shown in Fig. 1. The administration of LPS lead to the appearance of a significant population of $CD86^+$ cells in peritoneal cells and spleen cells. The frequency of $CD86^+$ cells in peritoneal cells and spleen cells was 37.9 and 33.7%, respectively (Fig.

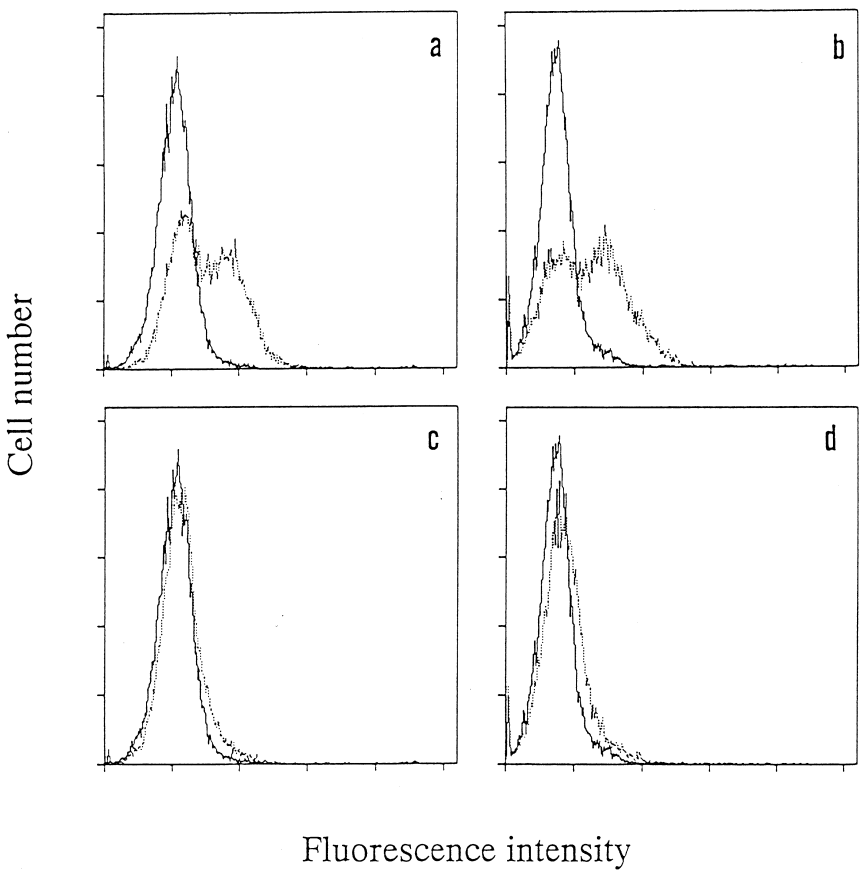

Fig. 1. Expression of CD86 on peritoneal cells and spleen cells from mice injected i.p. with LPS. Peritoneal cells (a,c) and spleen cells (b,d) from mice injected i.p. with LPS (100 μg) 18 h before (dotted line) or with PBS (solid line) were stained with an immunofluorescent technique using anti-CD86 antibody (a,b) or anti-CD80 antibody (c,d). The fluorescence intensity (x-axis) ranges logarithmically from 10^0 to 10^3. Relative cell number is expressed on the y-axis.

1a,b). CD86 was not detected on thymocytes and lymph node cells from mice injected i.p. with LPS. In addition, no significant CD80$^+$ cells were detected in cells from those organs of mice injected i.p. with LPS (Fig. 1c,d). Next, the expression of CD86 was studied by an immunohistochemical staining. CD86 was exclusively detected in the white pulp of spleens from mice injected with LPS, while it was hardly detectable in the red pulp and the marginal zone (Fig. 2). Some cells in the white pulp were strongly positive. On the other hand, only a few positive cells were scattered in the white pulp of spleens from mice injected with PBS.

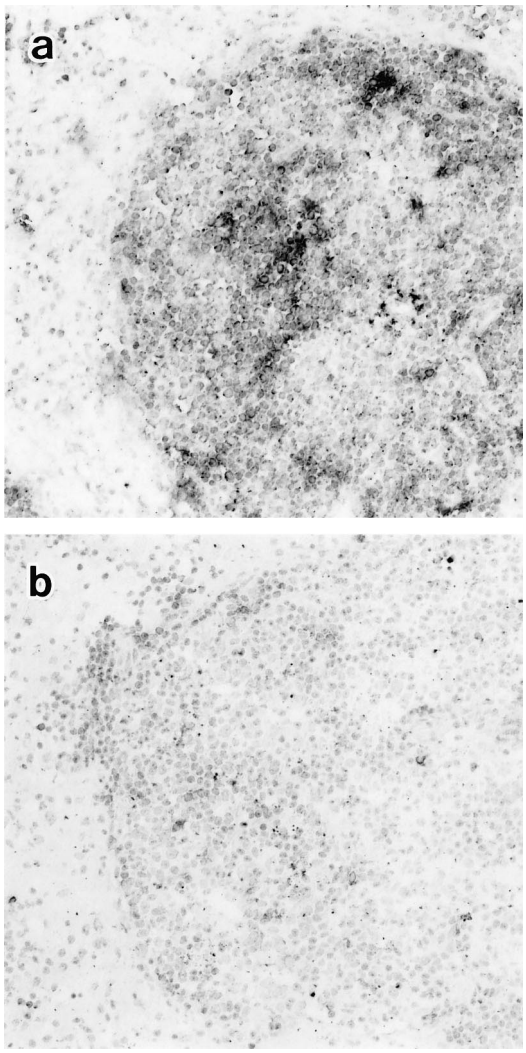

Fig. 2. Immunohistochemical staining of CD86 in spleens of mice injected i.p. with LPS. Spleens from LPS-injected mice (a) or PBS-injected control (b) were stained by anti-CD86 antibody. Note positive staining in the white pulp of the spleen (a).

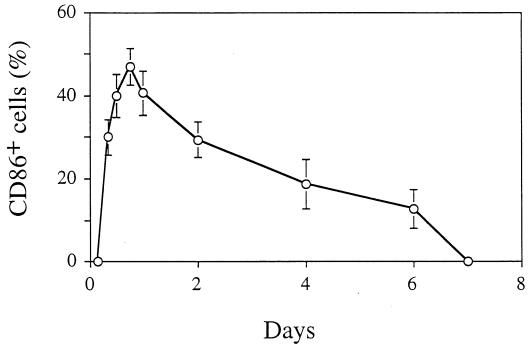

Fig. 3. Time course in the induction of peritoneal CD86$^+$ cells by administration of LPS. The frequency of peritoneal CD86$^+$ cells with SD in mice injected i.p. with LPS (100 μg) was analyzed by a laser flow cytometry.

3.3. Dose dependency of LPS injected on expression of CD86 on peritoneal cells

To investigate the dose effect of LPS injected on induction of CD86$^+$ cells in the peritoneal cavity, mice were injected i.p. with various doses of LPS. Peritoneal cells were isolated 16 h later after the injection, and the appearance of CD86$^+$ cell in the peritoneal cavity was studied with the flow cytometry. The experimental result is shown in Table 2. CD86$^+$ cells appeared in the peritoneal cavity of mice injected with more than 10 μg of LPS, and there was no significant difference in the frequency of CD86$^+$ cells between 10 and 100 μg. On the other

Table 2
Dose dependency of LPS injected on expression of CD86 in peritoneal cells and spleen cells

LPS injected (μg)	CD86 (% positive ± S.D.)	
	Peritoneal cells	Spleen cells
0	0	0
0.1	0	0
1	0	0
10	37.8 ± 4.3	30.7 ± 5.2
100	39.9 ± 5.0	34.7 ± 6.3

Mice were injected i.p. with various doses of LPS, and 16 h later, the expression of CD86 on peritoneal cells and spleen cells was estimated with the laser flow cytometry.

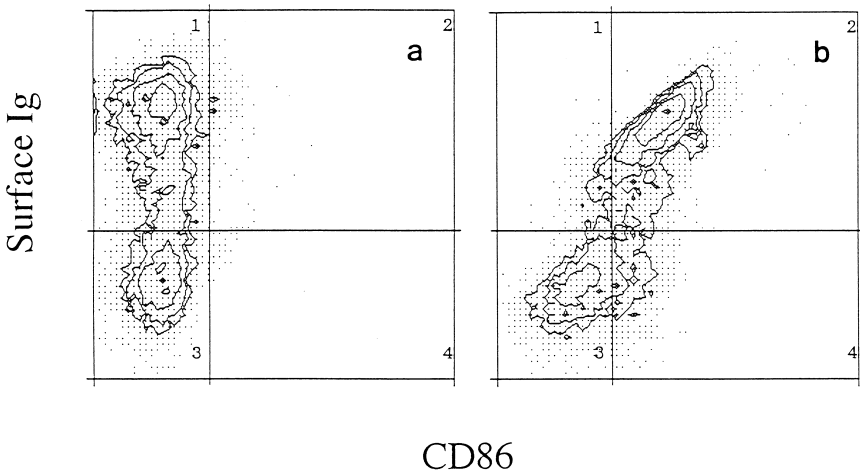

Fig. 4. Cell types of peritoneal CD86$^+$ cells by i.p. administration of LPS. Peritoneal cells from mice injected i.p. with PBS (a) or with LPS (100 μg) 18 h before (b) were stained by anti-CD86 antibody and anti-mouse Ig antibody. The green (x-axis) and red fluorescence intensity (y-axis) ranges logarithmically from 10^0 to 10^3.

hand, the injection of less than 1 μg of LPS did not induce CD86$^+$ cells in the peritoneal cavity. It should be noted that the injected dose of LPS for induction of CD86 expression was consistent with that required for triggering of IgE antibody response.

3.4. Time course in appearance of peritoneal CD86$^+$ cells by i.p. administration of LPS

The time course of appearance of CD86$^+$ cells in the peritoneal cavity of mice injected with LPS (100 μg) was followed. The experimental result is shown in Fig. 3. CD86$^+$ cells were detected in the peritoneal cavity 8 h after the injection of LPS, and increased up to 18 h. The frequency of peritoneal CD86$^+$ cells reached the peak at 18–24 h, and it gradually decreased. CD86$^+$ cells disappeared approximately 8 days after LPS injection. The time course of the appearance of CD86$^+$ cells in spleen cells was almost the same as that of peritoneal cells (data not shown).

3.5. Induction of peritoneal CD86$^+$ cells by administration of various preparations of LPS

The induction of CD86$^+$ cells in the peritoneal cavity was compared by administration of various preparations of LPS. Mice were injected i.p. with various preparations of LPS (100 μg), and 16 h later, the frequency of peritoneal CD86$^+$ cells was estimated. The experimental result is shown in Table 3. CD86$^+$ cells were detected in the peritoneal cavity of mice injected with LPS from *E. coli* O111, *E. coli* O55 or *S. enteritidis* and there was no significant difference in the induction of CD86$^+$ cells among those LPS preparations. Furthermore, we studied the effect of the free lipid A, O-specific polysaccharide, rough type LPS and smooth type LPS from *K. pneumoniae* O3 on the induction of CD86$^+$ cells in the peritoneal cavity. The injection of the free lipid A and rough-type LPS definitely caused the appearance of CD86$^+$ cells, while that of O-specific polysaccharide did not cause it. It was suggested that the active

Table 3
Induction of peritoneal CD86$^+$ cells by i.p. administration of various preparations of LPS

Preparation	CD86 (% positive ± S.D.)
None	0
E. coli O111 LPS	37.7 ± 6.2
E. coli O55 LPS	30.3 ± 4.8
S. enteritidis LPS	29.7 ± 2.0
Klebsiella O3 LPS	32.9 ± 3.9
Rough-type LPS	43.4 ± 5.8
Free lipid A fraction	46.3 ± 5.7
O-specific polysaccharide fraction	0

Mice were injected i.p. with various preparations of LPS (100 μg), and 16 h later, the expression of CD86 on peritoneal cells was estimated with the laser flow cytometry.

site of LPS for the induction of peritoneal CD86$^+$ cells was the free lipid A moiety.

3.6. Cell type of peritoneal CD86$^+$ cells by i.p. administration of LPS

Cell type of CD86$^+$ cells in the peritoneal cavity was studied by the flow cytometric analysis using two-color immunofluorescent staining. Peritoneal cells from LPS-injected mice were stained with FITC-conjugated anti-CD86 antibody and PE-conjugated anti-mouse Ig antibody. Most of surface Ig B-cells co-expressed CD86 (Fig. 4). Most of CD86$^+$ spleen cells was Ig B-cells. An Ig$^-$ cell population contained some CD86$^+$ cells, which were adherent cells.

3.7. Expression of CD28 and CTLA-4 by in vivo administration of LPS

Expression of CD28 and CTLA-4, possible ligands of CD86 [1–3,7], in mice injected with LPS was studied by the laser low cytometry. Mice were injected i.p. with LPS (100 μg). One or 3 days later, spleen cells and peritoneal cells were prepared from LPS-injected mice, and they were stained with an indirect immunofluorescence method using anti-CD28 or CTLA-4 antibody. The frequency of CD28$^+$ cells significantly decreased in the peritoneal cavity 1 and 3 days after the injection, whereas it did not alter in spleen. On the other hand, an i.p. administration of LPS did not affect expression of CTLA-4 on peritoneal cells and spleen cells.

4. Discussion

In the present study, we demonstrated that an i.p. injection of anti-CD86 antibody inhibited anti-ascaris extract IgE antibody response by LPS. Therefore, the expression of CD86 molecule might play a critical role in triggering of antigen-specific IgE antibody production by LPS. In fact, CD86 is known to possess the ability to potentiate the differentiation of T-helper cells into Th2 cells which support a humoral immune response [3]. It was, therefore, reasonable that in vivo expression of CD86 was required for triggering and differentiation of ascaris extract-specific helper T-cells in antigen-specific IgE antibody production. This idea is also supported by recent evidence that CD86 co-stimulates the initial production of Th2-associated cytokine, IL-4 [24]. IL-4 secretion must be conducted in vivo by Th2 cells since the development of antigen-specific IgE response has been shown to be entirely dependent upon production of IL-4 [25,26]. Once again, LPS-induced CD86 expression might play a critical role on triggering of Th2 cells producing IL-4 for antigen-specific IgE antibody production.

The i.p. administration of LPS into mice lead to the expression of CD86 on peritoneal cells and spleen cells. CD86$^+$ cells were B-cells and a part of macrophages. This is the first report that an in vivo administration of LPS into mice resulted in CD86 expression on B-cells and macrophages in spleen cells and peritoneal cells, although it was reported that the injection of LPS upregulated CD86 expression in splenic dendritic cells [18]. On the other hand, the in vitro stimulation with LPS has been reported to induce the expression of CD86 on multiple cell types, including mouse B-cells, macrophages and dendritic cells [8,9,15–17]. Our finding in an in vivo system was almost consistent with the expression of CD86 in in vitro systems. It should be noted that the injection of LPS did not result in detectable expression of CD80 on those cells, although CD80 is reported to be expressed on mouse B-cells and human monocytes stimulated in vitro by LPS [8,15,27,28]. It was likely that the in vivo expression of CD80 and CD86 might be differentially regulated by LPS [29].

Cell types of CD86$^+$ cells seemed to be mainly B-cells on the basis of two-color flow cytometric analysis. LPS was suggested to induce in vivo activation of B-cells and subsequently CD86 expression on them. This might be related to the ability of LPS to activate mouse B-cells polyclonally. Our in vivo finding was consistent with the fact that CD86 is rapidly induced on the surface of LPS-activated B-cells in an in vitro system [8]. Furthermore, it was supported by the fact that CD86 is originally defined as a B-cell activation marker [7]. Selected CD86$^+$ B-cells would process and present antigen to CD4$^+$ helper T-cells, which trigger antigen-specific IgE antibody response.

LPS has been shown to exhibit a novel adjuvant action through inhibiting the induction of tolerance

with non-immunogenic tolerogenic antigen [30–32]. Interaction of naive T-cells with non-immunogenic antigen in the presence of insufficient co-stimulatory signal may result in T-cell unresponsiveness or tolerance [2,3]. LPS-induced CD86 expression on antigen-presenting cells, such as B-cells and macrophages, might be essential to prevent tolerance induction and to trigger immune responses to tolerogenic antigen. The induction of CD86 expression on antigen-presenting cells by LPS may be associated with its novel adjuvant action to prevent the induction of tolerance with non-immunogenic tolerogenic antigen.

Recently, it has been reported that CD86/CD28 and CTLA-4 co-stimulatory pathways are required for the development of Th2-mediated allergic response to antigens [33,34]. In fact, our results demonstrated that the blockage of CD86 interactions effectively abrogates the potentiation of IgE antibody response by LPS. This supported that CD86 expression on antigen-presenting cells might play an immunopathogenic roles in the development of allergic diseases. Interestingly, our finding suggested the possibility that LPS might initiate and regulate Th2-mediated allergic responses through augmented CD86 expression. The role of LPS in the triggering of allergic response to various allergens should be clarified.

Acknowledgments

This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan. We are grateful to Makoto Naruse, Akiko Morikawa, and Kazuko Takahashi for technical assistance.

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