



Effects of CD80 and CD86 on cytokine production in patients with wasp-venom allergy who receive venom immunotherapy

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Abstract

Several studies have provided evidence that activation of antigen-specific T cells requires interactions between CD28 on T cells and its ligands, CD80 and CD86, on antigen-presenting cells (APCs). However, the effects of CD80 and CD86 on cytokine production in patients with Hymenoptera venom allergy who receive venom immunotherapy remain unclear. We examined the effects of CD80 and CD86 on Th1- and Th2-cytokine production before and after venom immunotherapy in patients with wasp-venom allergy. Peripheral blood mononuclear cells (PBMCs) were isolated from patients with wasp-venom allergy before and after three months of venom immunotherapy. CD4⁺ T cells and monocytes were isolated as APCs from PBMCs and were cocultured with wasp venom in the presence of anti-CD80 or -CD86 blocking antibodies. Interleukin (IL)-4, IL-10, and interferon (IFN)- γ were measured by enzyme-linked immunosorbent assay. The expression of CD80 and CD86 on CD14⁺ PBMCs was detected by fluorescence-activated cell-sorter analysis. The expression of CD86, but not that of CD80, on CD14⁺ PBMCs cocultured with venom increased after three months of venom immunotherapy, but not before venom immunotherapy. Blockade of CD86 reduced IL-10 production after three months of venom immunotherapy. IL-10 production promoted by CD86 costimulation may be involved in the mechanism of venom immunotherapy in patients with venom allergy.

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Keywords: Venom immunotherapy; Wasp-venom allergy; CD80; CD86; Interleukin-10

Abbreviations: Ag, antigen; APCs, antigen-presenting cells; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; mAb, monoclonal antibody; PBMCs, peripheral blood mononuclear cells

1. Introduction

T cells producing the T helper (h) 2 cytokines interleukin (IL)-4, IL-5, IL-9, and IL-13 are increased in patients with allergic diseases [1–3] and increase further after allergen challenge [4]. These T cells are thought to orchestrate immune and inflammatory processes that lead to IgE production and allergic in-

flammation or that accompany tissue injury. T cells also have an important part in Hymenoptera allergy by modulating the release of Th2 cytokines (IL-4, IL-5, IL-13) [5,6]. Venom immunotherapy in patients with Hymenoptera allergy induces IL-10 and interferon (IFN)- γ production by CD4⁺ T cells and monocytes, respectively, as well as a Th2-to-Th1 shift [7,8]. The activation of T cells participating in primary immune responses requires at least two signals from antigen-presenting cells (APCs): the first is provided by the cognate interaction of the T-cell receptor with MHC II complexes on APCs and the second by CD80 (B7-1) and

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CD86 (B7-2) on APCs interacting with CD28 receptors on T cells [9,10]. Several studies have reported that costimulation by CD86 is involved in airway APC and T-cell responses to allergens in patients with atopic asthma [11,12]. Recent investigations suggest that costimulation by CD80 expressed by alveolar macrophages is involved in allergen responses in the airways of asthmatic subjects [13]. In a murine model of contact sensitivity, anti-CD86 monoclonal antibody (mAb) improved disease only if administered before sensitization, while anti-CD80 mAb had no effect [14]. Thus, costimulation by CD80 and CD86 plays an important role in T-cell activation in allergic disease. However, the effects of CD80 and CD86 on cytokine production in patients with Hymenoptera allergy before and after venom immunotherapy remain unknown. In this study, we examined whether CD28-B7 costimulation was required for allergen-induced cytokine production by peripheral blood mononuclear cells (PBMCs) before and after venom immunotherapy in patients with wasp-venom allergy.

2. Results

2.1. Expression of CD80 and CD86 on CD14⁺ PBMCs before and after three months of wasp-venom immunotherapy

The expression of CD80 and CD86 was examined on the basis of mean fluorescence intensity (MFI). Before venom immunotherapy there was no change in the MFI of CD80 or CD86 expression on CD14⁺ PBMCs in the presence of wasp venom as compared with the expression of these molecules in the absence of venom (Fig. 1A, B). After three months of venom immunotherapy, the MFI of CD86 expression on CD14⁺ PBMCs in the presence of wasp venom significantly increased from 54.2 ± 44.7 to 123.3 ± 23.6 at 48 h ($p < 0.05$) and from 50.2 ± 31.5 to 90.1 ± 7.9 at 72 h ($p < 0.05$). The MFI of CD80 expression at 48 and 72 h was unaffected by stimulation with venom after three months of venom immunotherapy.

2.2. Effects of CD80 and CD86 on cytokine production in cultures of CD4⁺ T cells and monocytes before and after three months of wasp-venom immunotherapy

To examine the effects of CD80 and CD86 costimulatory molecules on cytokine production, anti-CD80 or anti-CD86 mAbs were added to cultures of CD4⁺ T cells and monocytes. As shown in Fig. 2, wasp-induced IL-4 and IFN- γ production was not reduced by inhibition of CD80 or CD86 before or after three months of venom immunotherapy. IL-10 production after three months of venom immunotherapy, but not before venom immunotherapy, was significantly reduced from 98.6 ± 17.7 to 58.6 ± 16.3 pg/ml by blockade of CD86 ($p < 0.05$).

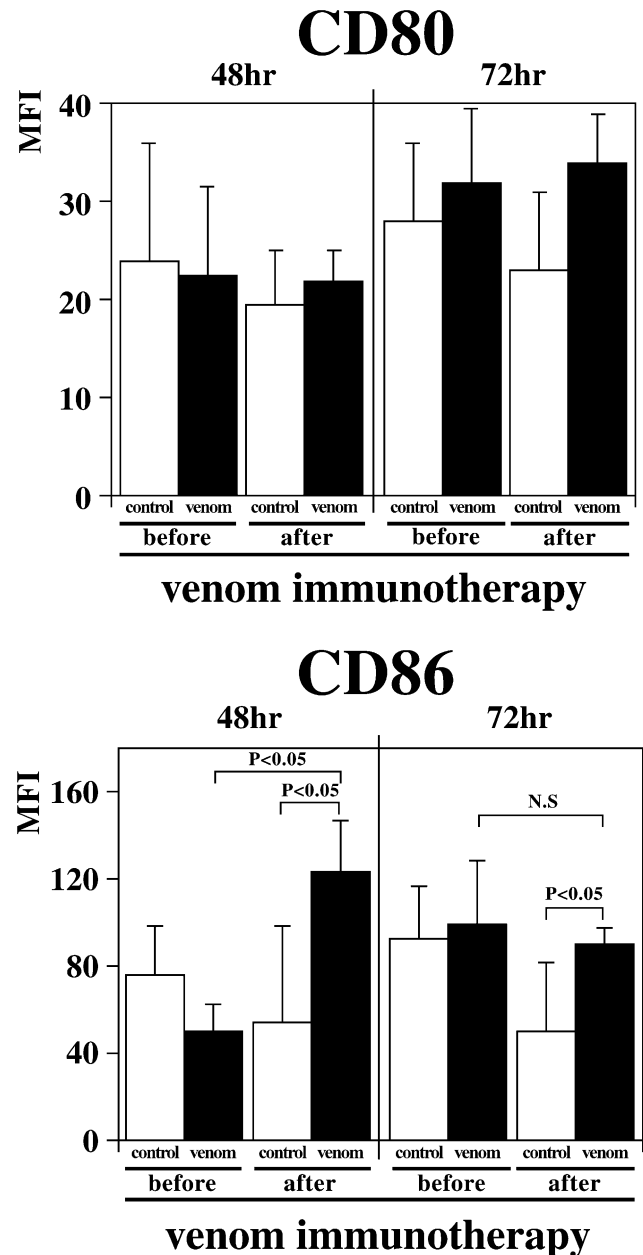


Fig. 1. Expression of CD80 (A) and CD86 (B) on CD14⁺ PBMCs stimulated with wasp venom before and after three months of venom immunotherapy. MFI values are means $\pm$ SD for five patients. PBMCs were cultured with wasp venom for 48 and 72 h. $p < 0.05$ as compared with between the expression of CD80 or CD86 by stimulation with venom before and after venom immunotherapy, or between unstimulated and stimulated venom at each time. NS, non-significance.

Blockade of CD80 had no discernible effect on IL-10 production.

3. Discussion

Interactions between CD28 on T cells and its ligands, CD80 and CD86, on APCs play an important role in

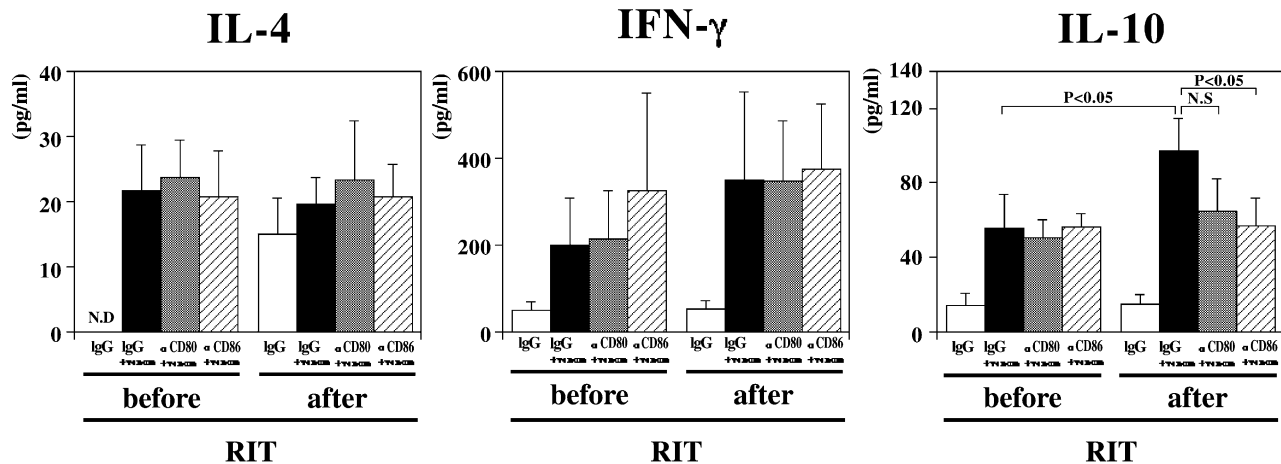


Fig. 2. Effects of CD80 and CD86 on allergen-driven production of IL-4, IL-10, and IFN- γ before and after three months of venom immunotherapy. Values are means $\pm$ SD for five patients. CD4⁺ T cells and monocytes were cultured with wasp venom for 48 h. $p < 0.05$ as compared with between stimulated venom before and after venom immunotherapy, or between stimulated venom and a blockade CD80 or CD86 at each time. NS, non-significance.

activation of antigen-specific T cells [9–12]. In Hymenoptera allergy, antigen-specific T cells stimulated by venom, acting as antigen, produce Th2 cytokines (IL-4, IL-5, and IL-13) [5,6]. Venom immunotherapy increases the number of IL-10-producing CD4⁺ T cells among PBMCs by antigen stimulation [7,8]. Our study showed that blockade of either CD80 or CD86 did not alter IL-4, IL-10, or IFN- γ production before venom immunotherapy. However, after three months of venom immunotherapy, the expression of CD86 on CD14⁺ PBMCs, but not that of CD80, increased in the presence of venom. Inhibition of CD86 reduced IL-10 production in our culture system.

Several studies have reported that CD86 costimulation is involved in airway APC and T-cell responses to allergens in allergic diseases [9–12]. We demonstrated that the expression of CD80 and CD86 on CD14⁺ PBMCs was unchanged in the presence of wasp venom before venom immunotherapy. We also found that antigen induced IL-4, IL-5, and IFN- γ production in our culture system; blockade of either CD80 or CD86 did not reduce antigen-induced production of IL-4, IL-10, or IFN- γ by CD4⁺ T cells, monocytes, or both. Th1/Th2 cytokines are generally produced by memory/effector T cells, including antigen-specific T cells, but not by naive T cells. Signaling by costimulatory molecules such as CD28 is more critical for the optimal activation of naive T cells than for the activation of memory cells [17,18]. Therefore, cytokine production by activated memory/effector T cells via CD28/CD80 or CD86 may be weaker than that by activated naive T cells, because Th2-cytokine-producing cells in patients with venom allergy before venom immunotherapy were primarily memory/effector T cells. Peng et al. reported that Th1- and Th2-cytokine production plays an important role in CD40/CD40L interactions [19].

Hintzen et al. showed that T-cell activation participates in CD27/CD70 interaction [20]. Other studies have similarly reported that B7.2 costimulatory molecules play an essential role in the induction of low-dose, but not high-dose, oral tolerance [21]. These findings indicate that cytokine production by activated CD4⁺ T cells in Hymenoptera allergy may be mediated by pathways involving other costimulatory molecules and may be affected by antigen concentration.

IL-10 production in response to venom immunotherapy induces anergy of peripheral T cells, affects the production of Th1 and Th2 cytokines, and may play an important part in immune mechanisms regulating antigen-specific T-cell responses [4]. We showed that the levels of IFN- γ and IL-10 after three months of venom immunotherapy were higher than those before venom immunotherapy. In addition, we found that monocytes, but not CD4⁺ T cells, by stimulation with venom extracts in normal subjects and in patients with wasp-venom allergy before venom immunotherapy could produce a small amount of IL-10 (data not shown). These results show that activated monocytes before venom immunotherapy and in the presence of anti-CD86Ab after venom immunotherapy may induce venom immediate IL-10 production. Furthermore, other studies have reported that increased production of IL-10, but not of IFN- γ , from CD4⁺ T cells in response to immunotherapy causes specific anergy of peripheral T cells [7,8]. Magnan et al. have shown that venom immunotherapy induces monocyte activation [8]. These findings indicate that venom immunotherapy induces antigen immediate-IFN- γ production by monocytes and suggest that antigen-specific CD4⁺ T cells produce IL-10.

In our study, the expression of CD86, but not of CD80, on CD14⁺ PBMCs was enhanced by stimulation

with wasp venom after three months of venom immunotherapy. The expression of CD86 after venom immunotherapy at each time point significantly ($p < 0.05$) increased by stimulation with venom extracts, however, the level of CD86 at 72 h tends to decrease compared with that of CD86 at 48 h. On the other hand, the level of CD86 before venom immunotherapy at 72 h showed a tendency to increase compared with that of CD86 at 48 h. These results indicate that time kinetics on the expression of CD86 may be altered by venom immunotherapy. Moreover, Larsen et al. reported that CD86 expression increases in dendritic cells cocultured with IFN- γ [22]. Adrienne et al. found that expression of CD86, but not of CD80, increases in activated CD4⁺T cells [23]. Therefore, increased expression of CD86 by stimulation with antigen may be involved in IFN- γ production and induction of decreased IL-10 production by venom immunotherapy. We also showed that the blockade of CD86, but not of CD80, reduced IL-10 production after three months of venom immunotherapy. Several studies have demonstrated that T cells activated by CD28 costimulation produce IL-10 [24,25]. However, CD28 expression is increased in patients with venom allergy but is not modified by specific immunotherapy [26]. Interestingly, other studies have shown that anergic cells produce high levels of IL-10 [27]. Furthermore, a recent study demonstrates that the specific effector function of allergen-specific T cells is modulated by altered TCR ligands [23]. These findings indicate that venom immunotherapy may regulate signals via CD28/CD86 on IL-10-producing-anergic T cells, but not affect Th1- or Th2-cytokine-producing cells. Thus, IL-10 production by T cells activated via CD86 on monocytes may play an important role in the mechanism of venom immunotherapy.

4. Materials and methods

4.1. Subjects

Five patients with wasp-venom allergy who met the following eligibility criteria were enrolled: (1) a history of generalized reactions of Muller grade [15] ≥ 1 , (2) positive cutaneous reactions to wasp venom (Bayer Corporation, USA), (3) wasp-venom-specific IgE antibody in sera as detected by RAST (RAST score $\geq$ class 2), and (4) a positive skin test response to wasp venom (≤ 1 μ g/ml venom injected intracutaneously) (Table 1). Informed consent was obtained from all patients before enrollment.

4.2. Immunotherapy protocol

Rush immunotherapy was performed with venom extract according to the protocol described by Lesourd

Table 1

Clinical features of patients with wasp-venom allergy

Patients	Sex (M/F)	Age (yr)	Grade	Specific IgE RAST (IU/L)
1	M	45	IV	3.8
2	M	49	IV	4.2
3	M	68	II	1.9
4	M	32	I	2.3
5	F	69	III	1.2

Grade, according to the method of Muller [15].

et al. [16] with slight modification. All patients were hospitalized during rush immunotherapy and received four subcutaneous injections per day of increasing doses of wasp-venom extracts (Bayer Corporation, USA), given at 2-h intervals. The dose of the first injection was determined on the basis of skin test reactions to the venom extracts. When the maximal dose of 100 μ g had been reached, the patients were given a maintenance dose of 100 μ g per venom at four- to six-week intervals.

4.3. Cell preparation

Peripheral blood was obtained from the subjects by venipuncture before and after three months of venom immunotherapy. Heparin was used as anticoagulant. PBMCs were separated by Ficoll–Hypaque density gradient centrifugation (Pharmacia, Uppsala, Sweden). Cells were washed three times with phosphate-buffered saline (PBS) supplemented with 2% fetal calf serum (FCS) and were resuspended at a concentration of 1×10^6 /ml. CD4⁺T cells and monocytes were purified from the PBMCs by negative selection, using mAbs that were bound in bispecific antibody complexes directed against cell-surface antigens on human hematopoietic cells and dextran. These cells are designed to be used with Stemsep TM Magnetic Colloid (StemCell Technologies Inc, Vancouver, Canada) in magnetic cell-separation studies. The purity of these cell preparations was checked by immunofluorescence, using anti-CD4 mAb, anti-CD19 mAb, and anti-CD14 mAb (Pharmingen, San Diego, CA) as CD4⁺T cells, B cells, and monocytes, respectively. The purity of each cell preparation was greater than 95%.

4.4. Cell culture

To determine CD80 and CD86 expression by FACS analysis, PBMCs (1×10^6 /ml) were cultured in 48-well plates in RPMI 1640 (Sigma, Poole, UK) medium supplemented with 10% FCS at 48 and 72 h. All tissue culture media and supplements were endotoxin-free. Cells were stimulated with wasp venom, concentrated to 10 μ g/ml, at 37 °C in a 5% CO₂ environment.

To determine IL-4, IL-10, and IFN- γ production, CD4⁺T cells and monocytes at a ratio of 1:1 were

cultured in 48-well plates in RPMI 1640 medium supplemented with 10% FCS. Cells were incubated at 37 °C in a 5% CO₂ environment for 30 min with anti-CD80 mAb (10 µg/ml), anti-CD86 mAb (10 µg/ml) (Pharmingen, San Diego, CA), or anti-mouse Ig (5 µg/ml) (Pharmingen, San Diego, CA), the latter serving as control. Cells were subsequently stimulated with wasp venom, concentrated to 10 µg/ml, at 37 °C in a 5% CO₂ environment. After 48 h of culture, the supernatants were harvested and stored at –80 °C until analysis. Because insect venoms contain cytotoxic components, the remaining cells were harvested and analyzed for viability by the trypan blue method.

4.5. Determination of surface marker expression and cytokine production by flow cytometry

Surface phenotyping of PBMCs was performed by staining 1×10^6 cells with marker-specific mAb for 30 min at 4 °C. The antibodies used were PE-conjugated anti-CD14, FITC-conjugated anti-CD80 (B7-1), and FITC-conjugated anti-CD86 (B7-2; Pharmingen, San Diego, CA). After staining in PBS supplemented with 2% FCS and 0.7% NaH, cells were analyzed on a FACScan (Becton Dickinson) equipped with Cell Quest software.

4.6. Cytokine assays

IL-4, IL-10, and IFN- γ concentrations in culture supernatants were assessed by enzyme-linked immunosorbent assay (ELISA) with Cytoscreen Immunoassay kits (Biosource, Inc., Camarillo, CA). The sensitivities of all assays were higher than 7.8 pg/ml.

4.7. Statistical analysis

Data are expressed as means $\pm$ SD. The statistical significance of differences between groups was examined by analysis of variance with Bonferroni's test. The *p* values of less than 0.05 were considered to indicate statistical significance.

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