

CD80 (B7-1) and CD86 (B7-2) antigens on house dust mite-specific T cells in atopic disease function through T-T cell interactions

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Background: CD80 (B7-1) and CD86 (B7-2) play an important role in antigen presentation to effector cells. Recent studies have demonstrated that these costimulatory molecules are also expressed on activated T cells. However, the functional role of CD80 and CD86 expressed on allergen-specific T cells in atopic diseases has not yet been clarified.

Objective: We sought to determine the functional role of CD80 and CD86 expressed on allergen-specific T cells in atopic diseases.

Methods: We assayed the expression of CD80 and CD86 on allergen-specific T-cell lines from patients with perennial allergic rhinitis stimulated by *Dermatophagoides farinae*-crude (Der f-c) antigen, 1 of the major allergens causing house dust mite allergy. T-cell proliferation induced by Der f-c-specific T-T cell interactions was measured, and the role of CD80 and CD86 in this proliferation was examined. In addition, we compared the proportion of CD45RO⁺CD86⁺ T cells in primary culture of PBMCs stimulated by Der f-c antigen between patients with perennial allergic rhinitis and control subjects. **Results:** On T-cell activation, CD86 antigen was upregulated earlier than CD80. Both CD80 and CD86 expressed on Der f-c-specific T cells could provide costimulatory signals to induce allergen-specific T-cell proliferation that was partially inhibitable by both anti-CD80 and anti-CD86 mAbs. The proportion of CD45RO⁺CD86⁺ T cells in primary culture from atopic patients was significantly higher than that from control subjects.

Conclusion: These results suggest that costimulatory molecules, such as CD80 and CD86, expressed on allergen-specific T cells may be involved in the amplification of allergen-specific immune responses through T-T cell interactions in atopic diseases. (*J Allergy Clin Immunol* 1999;104:222-7.)

Key words: Allergen-specific T cell, atopic disease, CD80, CD86, T-T cell interactions

Antigen-specific T cells require 2 distinct signals for functional activation.¹ The first signal is delivered by the

Abbreviations used

APC:	Antigen-presenting cell
CM:	Culture medium
Der f-c:	<i>Dermatophagoides farinae</i> -crude
PBMCx:	Autologous irradiated PBMC
PPD:	Purified protein derivative
TcR:	T-cell receptor

interaction of antigen/MHC with the T-cell receptor (TcR). The second signal is delivered by costimulatory molecules expressed on antigen-presenting cells (APCs). The binding of antigen/MHC to TcR in the absence of costimulatory molecules induces antigen-specific unresponsiveness or anergy.²⁻⁴ CD80 (B7-1) and CD86 (B7-2), members of the B7 family that bind to the counter-receptors CD28 and CTLA-4 on T cells, are 2 of the most important costimulatory molecules.⁵⁻⁷ CD80 and CD86 are generally expressed on APCs, such as macrophages, dendritic cells, Langerhans cells, and activated B cells.⁸ Recent studies have demonstrated that both CD80 and CD86 are also expressed on activated T cells in vitro and in vivo.⁹⁻¹⁵ Recently, it has been reported that CD80 molecules on CD4⁺ T-cell clones can stimulate a primary mixed lymphocyte reaction.¹⁰ Moreover, CD80 molecules on tetanus toxin-specific T-cell clones and on alloreactive CD4⁺ T-cell clones can provide costimulatory signals and induce T-cell proliferation through T-T cell interactions.^{11,13}

Atopic diseases are characterized by the immediate type of hypersensitivity. The production of allergen-specific IgE antibodies is crucial, and it is controlled by allergen-specific CD4⁺ T_H cells.¹⁶ IL-4 derived from T_{H2} cells upregulates the production of allergen-specific IgE antibodies, and it is suppressed by interferon- γ derived from T_{H1} cells.¹⁷ Allergen-specific T_{H2} cells predominantly proliferate in atopy,¹⁸⁻²¹ and their proliferation is triggered by the interaction between allergen-specific T cells and APCs. If allergen-specific T cells can also function as APCs by the expression of costimulatory molecules, they may be involved in long-lasting allergic immune responses. The expression of CD80 on anti-Der p 1 house dust mite-specific T-cell clone has been reported.¹⁰ However, the function and the role of CD80 and

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CD86 on allergen-specific T cells in atopic diseases have not yet been examined.

This study was designed to determine whether allergen-specific T cells can induce T-cell proliferation through T-T cell interactions. We assayed the expression of CD80 and CD86 on T-cell lines specific for *Der-matophagoides farinae*-crude extract (Der f-c) antigen. Using these cell lines from patients with perennial allergic rhinitis, we examined the role of CD80 and CD86 on allergen-specific T cells in T-cell proliferation. Results indicate that T-T cell interactions may function in atopic disease.

METHODS

Subjects

Ten Japanese patients (8 men and 2 women; age, 24 to 33 years) with perennial allergic rhinitis and 10 healthy individuals (8 men and 2 women; age, 25 to 40 years) without a history of atopic diseases were examined. Informed consent was obtained from each subject. All allergic patients showed elevation of anti-Der f-c-specific IgE antibodies in RASTs. None of the patients used immunosuppressive drugs or underwent immunotherapy during the study.

Antigens

Der f-c antigen, one of the major allergens of perennial allergic rhinitis caused by house dust mites,^{29,30} was provided by Torii Co (Tokyo, Japan). Purified protein derivative (PPD; Nihon BCG Co, Tokyo, Japan) was used as a negative or control antigen. The optimal concentration of each antigen for T-cell proliferation (10 to 100 µg/mL for Der f-c and 2 µg/mL for PPD) was determined in a preliminary experiment (data not shown).

T-cell lines

PBMCs were separated by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation from heparinized blood of 2 patients with perennial allergic rhinitis. To establish Der f-c-specific T-cell lines, PBMCs were stimulated with 10 µg/mL Der f-c antigen in RPMI-1640 culture medium (CM; Nikken Co, Kyoto, Japan) supplemented with 10% heat-inactivated pooled male human sera, 100 U/mL penicillin, 100 µg/mL streptomycin, and 20 mmol/L L-glutamine (Gibco, Grand Island, NY). After 7 days, the blastoid cells were stimulated again with 10 µg/mL Der f-c antigen, 20 U/mL human recombinant IL-2 (Boehringer Mannheim, GmbH, Germany), and autologous irradiated (30 Gy) PBMCs (PBMCx). CM containing IL-2 was changed every 3 days, and Der f-c antigen was added fortnightly.

Cell cultures

To examine the expression of CD80 and CD86 on allergen-specific T cells, established Der f-c-specific T-cell lines were restimulated with 10 µg/mL Der f-c antigen and cultured with autologous PBMCx in IL-2-supplemented CM; the kinetics of expression of these molecules was assessed by flow cytometry. For experiments that examined allergen-specific T-T cell interactions, long-term cultured T-cell lines were purified by a nylon-wool column 7 days after the eighth restimulation. The purity of blastoid cells was more than 98% CD3⁺. T-cell lines were treated with mitomycin C (Kyowa Hakko Kogyo Co, Tokyo, Japan); pulsed with various concentrations (1 to 100 µg/mL) of Der f-c antigen, PPD (2 µg/mL), or medium alone; and washed 4 times with 5% human sera-CM. Antigen or medium alone pulsed T-cell lines (1×10^5) used as APCs were cultured with 2×10^4 equivalent T-cell lines in 10% human sera-CM in 96-well, flat-bottom plates (Falcon Labware, Lincoln Park, NJ) for

72 hours at 37°C in 5% CO₂/95% air. Proliferation of T-cell lines was estimated by mean uptake of ³H-thymidine in triplicate cultures. For the inhibitory experiments, anti-CD80 mAb (clone BB1; Pharmingen, San Diego, Calif) and anti-CD86 mAb (clone IT2.2; Pharmingen), which contained no preservatives, were used; mixtures of these mAbs at concentrations of 1 to 100 µg/mL were added to the cultures described earlier. In the inhibitory experiments, Der f-c antigen was administered at the concentration of 100 µg/mL, which induced maximum proliferation of T-cell lines. For the comparison of CD80 and CD86 expression on Der f-c antigen- or PPD-stimulated T cells between patients with perennial allergic rhinitis and control subjects, 2×10^5 PBMCs were cultured in the presence or absence of 50 µg/mL Der f-c antigen or 2 µg/mL PPD for 9 days.

Flow cytometric analysis

The method of flow cytometry has been described elsewhere.²³ In brief, FITC-labeled anti-CD4, CD8, CD45RO, and HLA-DR/DQ/DP mAbs (DAKO Japan, Kyoto, Japan) and biotin-labeled anti-CD80 and B7-2 (CD86) mAbs (Pharmingen) were used as primary antibodies. The secondary antibody was phycoerythrin-labeled streptavidin (Ancell Co, Bayport, Minn). Biotin-labeled monoclonal mouse IgM (Ancell Co) and biotin-labeled monoclonal mouse IgG (Vector Laboratories, Burlingame, Calif) were used as negative controls for anti-CD80 and CD86, respectively. Stained samples were analyzed with FACSCalibur (Becton Dickinson, Mountain View, Calif). Der f-c antigen- or PPD-specific expression of CD80 and CD86 on T cells in PBMC primary culture was assessed by the calculation of the relative cell numbers (percentage) of CD45RO⁺CD86⁺ T cells in the presence or absence of antigen.

Statistical analysis

Data were recorded as antigen-specific relative cell number (percentage) as described earlier. Statistical comparisons were performed with the Student *t* test. A *P* value of less than .05 was considered statistically significant.

RESULTS

Differential expression of CD80 and CD86 on allergen-specific T cells

We established 3 Der f-c antigen-specific T-cell lines from patient 1 (1-1, 1-2, and 1-3) and 2 from patient 2 (2-1, and 2-2; Fig 1). Lines 1-1 and 2-1 were CD45RO positive and expressed both CD80 and CD86 after the fifth allergen restimulation. CD86 expression was detected 9 days after the first allergen stimulation, whereas CD80 expression was not found until the fifth allergen restimulation (data not shown). Mean fluorescence intensity of upregulated CD86 was higher than that of CD80 (Fig 1). These 2 molecules showed different kinetics of upregulation after allergen restimulation. CD86 expression upregulated somewhat earlier and peaked at 5 to 7 days after the fifth allergen restimulation, whereas CD80 expression upregulated later and peaked at 10 days (Fig 2). Upregulation of CD80 and CD86 expressed on allergen-specific T-cell lines was not detected after IL-2 stimulation only (data not shown). Other T-cell lines (1-2, 1-3, and 2-2) also showed similar expression of CD80 and CD86 after the restimulation with Der f-c, and therefore we used 1-1 and 2-1 cell lines for further experiments.

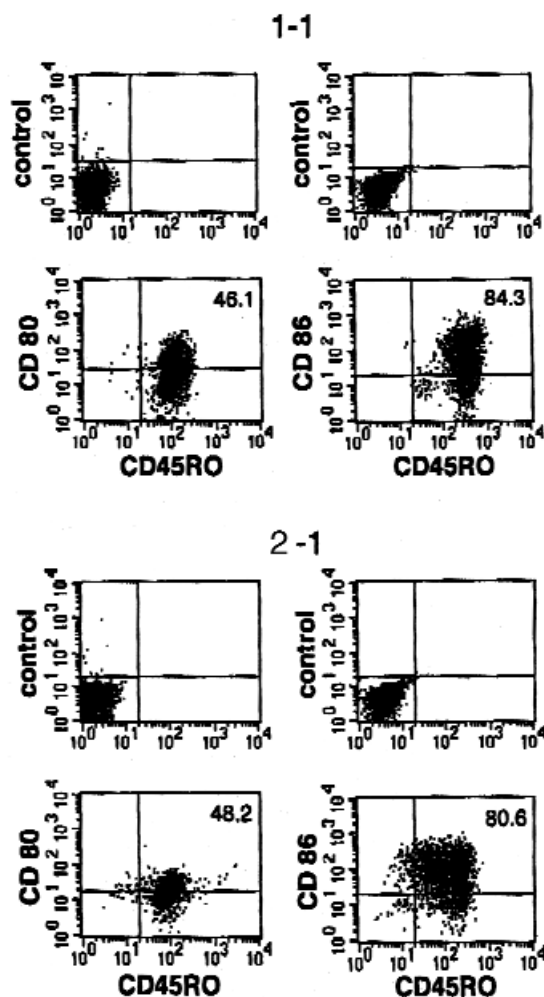


FIG 1. Flow cytometric analysis of the expression of CD80 and CD86 on Der f-c antigen-specific T-cell lines. Established Der f-c antigen-specific 1-1 and 2-1 cells from 2 patients with perennial allergic rhinitis were restimulated 5 times with Der f-c antigen (10 μ g/mL) and autologous PBMCx in IL-2 CM. Anti-CD45RO mAb was used as the marker of memory T cells. For the expression of CD80 and CD86, 1-1 cells were assessed at 10 and 7 days after allergen restimulation, and 2-1 were assessed at 10 and 5 days, respectively. Numbers in each of the dot plots show the relative cell number (%) of CD45RO⁺CD80⁺ or CD45RO⁺CD86⁺ T cells.

Allergen-specific T cells can induce T-cell proliferation

Long-term cultured 1-1 and 2-1 cells pulsed with Der f-c antigen at 7 days after the eighth restimulation induced allergen-specific proliferation of equivalent T-cell lines in a dose-dependent manner (Fig 3). 1-1 and 2-1 cells used in these experiments showed intense expression of HLA-DR/DQ/DP antigens (data not shown). The degree of this proliferation estimated by the uptake of ³H-thymidine was about one sixth of that found in cultures stimulated with autologous PBMCx (data not shown).

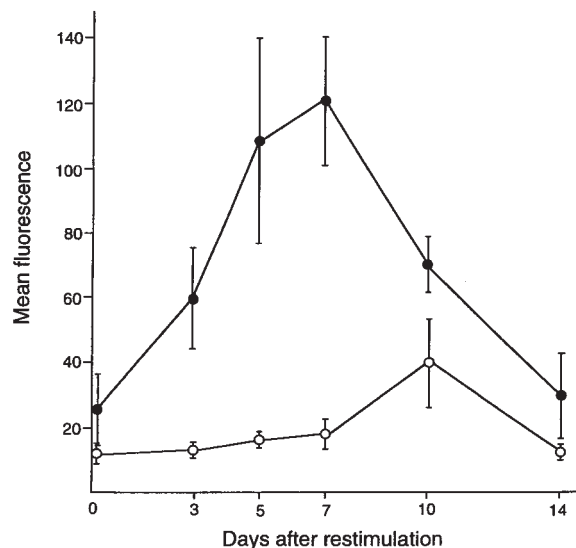


FIG 2. Kinetics of CD80 and CD86 expression on Der f-c-specific T-cell lines after the fifth allergen restimulation. Each T-cell line was cultured (see the Methods section) and analyzed after 3, 5, 7, 10, and 14 days by flow cytometry. Anti-CD45RO mAb was used as the marker of memory T cells. Results are shown in mean fluorescence intensity $\pm$ SD of CD80 (open circles) and CD86 (filled circles) after 3 individual experiments.

Functional involvement of CD80 and CD86 in T-cell proliferation induced by T-T cell interactions

Both anti-CD80 and anti-CD86 mAbs partially inhibited the T-T cell interaction-induced proliferation described earlier (Fig 4). The maximum inhibitory effects of anti-CD80 and anti-CD86 mAbs were approximately 40% and 50% at the highest dose tested. The maximum inhibitory effect of anti-CD86 mAb was slightly higher, although not statistically significant, than that of anti-CD80 mAb. Although the mixtures of these 2 mAbs showed greater inhibition, this augmentation was less than additive (data not shown).

Proportion of CD45RO⁺CD86⁺ T cells stimulated by allergen in atopic and control subjects

Antigen (Der f-c, PPD)-specific expression of CD80 and CD86 on T cells in PBMC culture was compared between the atopic and control subjects. No significant expression of CD80 and CD86 was detected on freshly isolated peripheral blood T cells (data not shown). Significant upregulation of CD86 was found in both groups after a single antigen stimulation (Fig 5), although no significant upregulation of CD80 was detected (data not shown). Antigen-specific T cells expressing CD86 were mainly CD4⁺CD45RO⁺ T cells; few CD8⁺CD86⁺ T cells were detected (data not shown). The proportion of CD45RO⁺CD86⁺ T cells stimulated with Der f-c antigen was significantly higher in the atopic subjects than that in

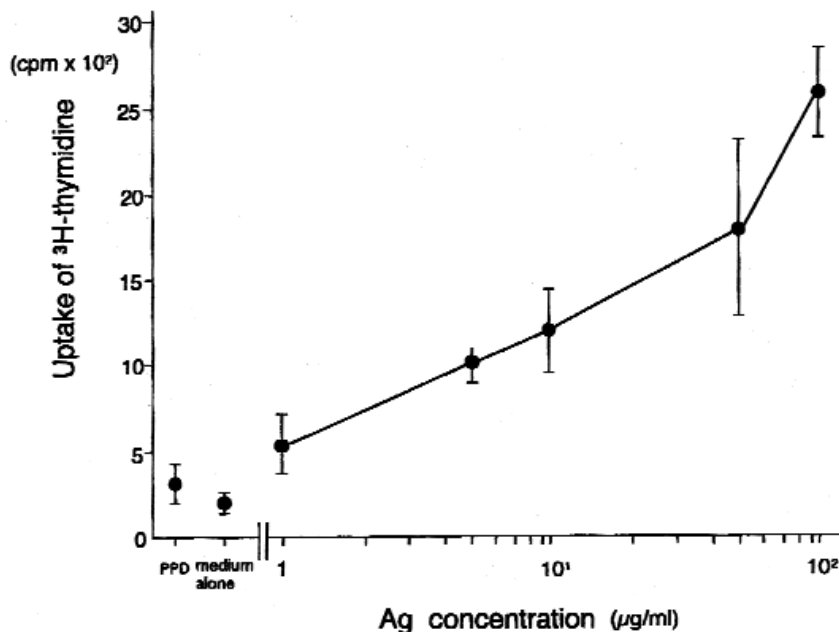


FIG 3. Dose-dependent proliferation of 1-1 cells in response to Der f-c antigen-pulsed 1-1 cells. 1-1 cells (1×10^5) treated with mitomycin C were pulsed with Der f-c antigen (1 to 100 $\mu\text{g/mL}$), PPD (2 $\mu\text{g/mL}$) or medium alone, and then cocultured with 2×10^4 equivalent 1-1 cells for 72 hours. Cell proliferation was estimated by mean uptake of ^3H -thymidine (counts per minute) in triplicate cultures. Three representative experiments were executed. Bars indicate means $\pm$ SD. Results were concordant with data from 2-1 line. Ag, Antigen.

the control subjects ($P < .05$), although no significant difference was seen in PBMC culture with PPD (Fig 6). The intensity of CD86 expression on Der f-c-specific T cells from the atopic patients was also higher than that from the control subjects (data not shown).

DISCUSSION

This study demonstrated that allergen-specific T cells expressed both CD80 and CD86 and that these 2 molecules could provide costimulatory signals to equivalent T cells and induce T-cell proliferation. CD86 on T cells was upregulated earlier on allergen stimulation, although CD80 on T cells was induced later. CD86 on T cells overexpressed by allergen restimulation also reached a peak value earlier than did CD80. These results were similar to those in former reports.^{9-11,22} In line with the suggestions,²³⁻²⁵ prompt and stronger upregulation of CD86 on T cells seemed to be more closely associated with the immune response; the patient-control study revealed that CD45RO⁺CD86⁺ T cells in PBMC culture stimulated by allergen were in greater proportion in patients with atopic disease than in normal subjects, and CD80 was not significantly upregulated in either group. This is the first report that T-T cell interaction involving the B7 family functions in atopic diseases. Because IL-2 stimulation alone could not induce significant upregulation of CD80 and CD86 on allergen-specific T cells, signaling through CD28 might be required for the induction of CD80 and CD86 on T cells as described before.⁹

Der f-c antigen-pulsed T cells expressing both CD80

and CD86 induced Der f-c-specific T-cell proliferation in a dose-dependent manner. It is known that the extract of Der f-c contains a low amount of endotoxin. However, our result is not due to a mitogenic effect of Der f-c because an addition of polymyxin B did not affect GM-CSF production by PBMC stimulated with the Der f-c provided by Torii Co.³¹ In addition, the data that the expression of CD86 in T cells cultured with Der f-c was different between healthy and allergic subjects (Fig 6) may support the negligible effect of endotoxin in Der f-c. Moreover, these cell lines did not react with PPD, suggesting that the lines were indeed specific for Der f-c. This proliferation was partially inhibitable by both anti-CD80 and anti-CD86 mAbs. These results suggest that allergen-specific T cells can capture, process, and present native allergens to other T cells and that both CD80 and CD86 on allergen-specific T cells can provide costimulatory signals to T cells. Wyss-Coray et al¹¹ and Barnaba et al¹³ reported similar findings with respect to CD80. Barnaba et al¹³ reported that activated T cells can capture soluble antigen by pinocytosis and that this capacity of T cells is closely associated with the synthesis of MHC class II antigens and upregulation of B7 molecules. Our results demonstrated that it took 7 days after allergen restimulation for both MHC class II antigens and B7 molecules to be upregulated in a manner capable of an antigen-presenting function. As T-T cell interaction-induced proliferation in this study was less than that induced by coculture with autologous PBMCx, the capacity of activated T cells for antigen processing is probably less than that by B cells or monocytes in PBMC. Some recent

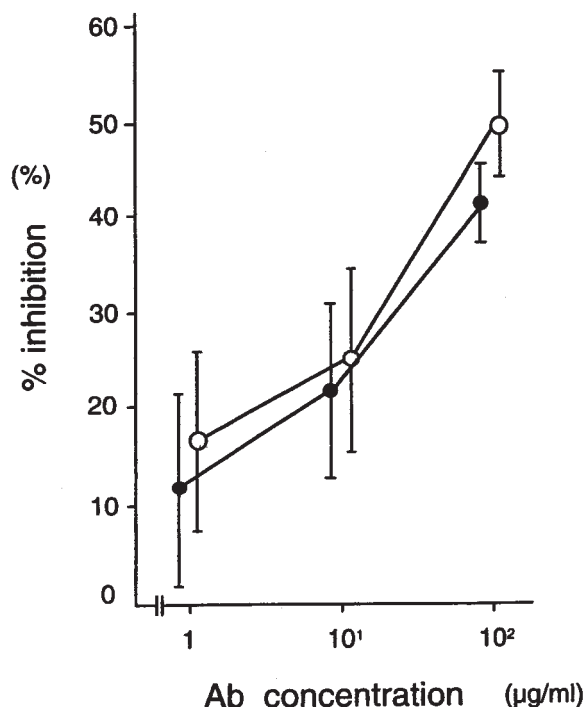


FIG 4. Inhibitory effect of anti-CD80 or anti-CD86 mAb on the proliferation of 1-1 cells induced by T-T cell interactions. Anti-CD80 mAb, anti-CD86 mAb, or their mixtures (1, 10, 100 µg/mL) were added to the 1-1 culture stimulated with mitomycin C-treated homologous cells pulsed with 100 µg/mL of Der f-c (see Fig 3). The inhibitory effects (percent of inhibition) of each molecule on T-T cell interaction-induced proliferation were estimated by the formula: $100 \times (\text{mean counts per minute in culture without mAb} - \text{mean counts per minute in culture with mAb}) / \text{mean counts per minute in culture without mAb}$. Filled circles represent anti-CD80 mAb. Open circles represent anti-CD86 mAb. Isotype-matched control antibody (Ab; mouse IgM and IgG2b for anti-CD80 and CD86, respectively) had little effect on the inhibition of proliferative responses by T-T interactions (data not shown). Results were concordant with data from the 2-1 line.

reports, however, have shown that CD86 on T cells preferentially binds to CTLA-4 and downregulates immune responses.^{15,26,27} Although the reason for this discrepancy has not been fully identified, it is possible that it relates to a difference of affinity, binding epitope, and range of doses of mAb used in blocking experiments, kinds of antigens, and strength of B7 expression on activated T cells.

Both anti-CD80 and anti-CD86 mAbs partially inhibited T-cell proliferation, but additive effects of these mAbs were not observed. This result suggests that some other costimulatory molecules on T cells, such as CD58 (LFA-3),¹⁵ are also involved in T-T cell interactions.

Recent reports have suggested that B7 molecules on activated T cells may play a part in autocrine costimulation,¹⁰ autoimmune disease,¹³ and HIV infection.¹¹ The greater expression of CD86 on allergen-specific T cells in the atopic subjects than in the control subjects may be partly responsible for the greater immune responses to allergens in atopic subjects.²⁸ In addition, we observed

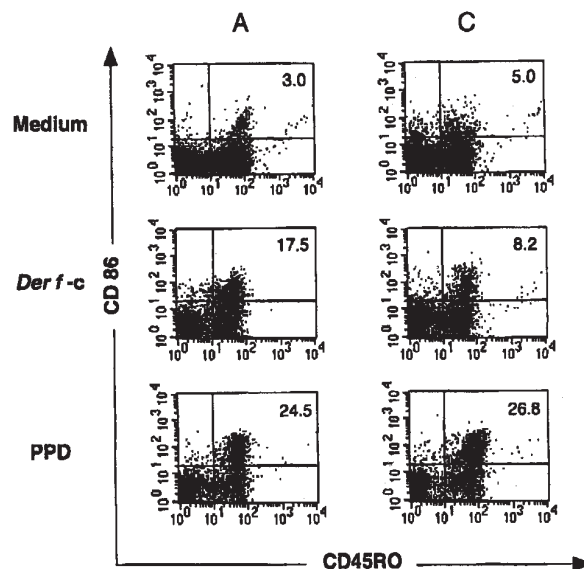


FIG 5. Flow cytometric analysis of CD45RO+CD86+ T cells in cultured PBMCs from atopic and control subjects stimulated with or without Der f-c antigen or PPD. PBMCs (2×10^5) from atopic and control subjects ($n = 10$ each) were cultured in the presence or absence of Der f-c antigen (50 µg/mL) or PPD (2 µg/mL) for 9 days followed by flow cytometric analysis. Data of a representative case from each group are shown. Numbers in each of the dot plots show the relative cell number (percent) of CD45RO+CD86+ T cells in PBMC culture. A represents the atopic subject; C represents the control subject.

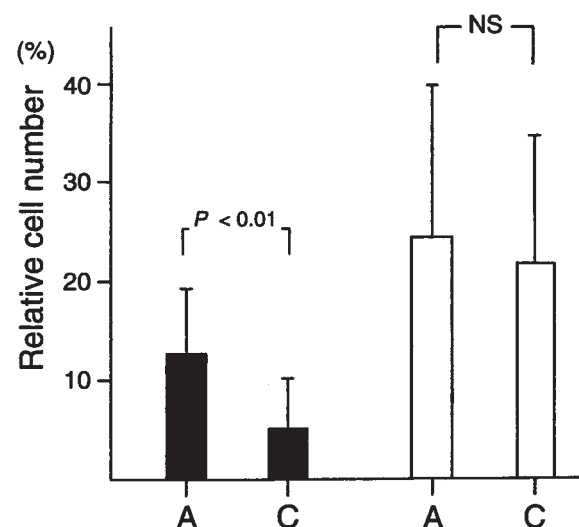


FIG 6. Relative cell number of CD45RO+CD86+ T cells in antigen-stimulated PBMC culture compared with antigen-free PBMC culture. The proportion of CD45RO+CD86+ T cells was estimated by flow cytometry (see Fig 5). Bars indicate means + SD. A represents atopic subjects; C represents control subjects. NS, Not significant. Solid bars represent the Der f-c antigen; open bars represent PPD.

the increase of CD28 expression on CD4+CD45RO+ T cells after in vitro culture of PBMC with Der f-c antigen in patients with rhinitis, whereas no increase was seen in healthy subjects. On the contrary, expression of CTLA-4

was not found in either atopic or nonatopic subjects (data not shown). It is known that ligation with CD28 mediates T-cell survival, whereas ligation with CTLA-4 induced cell death.³² Tsicopoulos et al³³ reported that CD28 expression was increased in venom-allergic patients compared with the control subjects. Our result is consistent with this report and might suggest that T cells from patients with rhinitis showed positive proliferation with the increased expression of CD28 after the stimulation with allergen. The role of other costimulatory molecules on T cells, such as CD58¹⁵ and CD40 ligand, in atopic diseases should be investigated in the future. Furthermore, blocking of T-T cell interactions might show promise as a therapeutic approach to atopic diseases.

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