

Expression of costimulatory CD80/CD86-CD28/CD152 molecules in nasal mucosa of patients with perennial allergic rhinitis

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Summary

Background B7 molecules (CD80, CD86) and their counter-receptors, CD28 and CD152 (CTLA-4), play an important role in T cell-mediated immune responses. We previously demonstrated that B7 molecules are selectively up-regulated not only on B cells but also on T cells from the peripheral blood mononuclear cells of patients with perennial rhinitis cultured with allergen. However, the expression of CD80/CD86 molecules and their counter-receptors in nasal mucosa, the actual inflammatory site of allergic rhinitis, has not yet been clarified.

Patients and methods Inferior turbinates from patients with either allergy to house dust or non-allergic rhinitis were excised and immunohistologically stained. In addition, the inferior turbinates were challenged with paper discs containing extracts of house dust and subsequently excised. Samples were double stained with immunofluorescent-labelled antibody to identify cells bearing CD86.

Results Without the nasal provocation, only the expression of CD86 was increased in nasal mucosa of patients with allergic rhinitis compared with those with non-allergic rhinitis. However, following the nasal provocation with house dust, not only CD86, but also CD80, CD28, and CD152 were significantly expressed in allergic patients. Immunofluorescent double staining revealed CD86 expression in CD19, CD1a, CD14 and CD3 lymphocytes.

Conclusion These results indicate that the expression of CD80/CD86 molecules and their counter-receptors is induced in allergic patients following nasal provocation with allergen, suggesting a local amplification of allergen-specific immune responses in perennial rhinitis.

Keywords: Costimulatory molecules, nasal mucosa, allergic rhinitis, nasal provocation

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Introduction

Allergic rhinitis is caused by immediate-type hypersensitivity associated with expansion of the Th2-type immune response and the production of allergen-specific IgE [1,2].

The activation, proliferation and cytokine production of antigen-specific T cells require two distinct signals from antigen-presenting cells (APC) [3,4]. The first signal is provided by the interaction of antigen/MHC with the T cell receptor (TcR). The second, called the costimulatory signal, is delivered by costimulatory molecules. Among these, the CD80/CD86 family CD80 (B7-1) and CD86 (B7-2) and their counter-receptors on T cells, CD28, CD152 (CTLA-4)

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are the most extensively characterized and appear to be the most crucial [5–7]. Signals through CD28 costimulate IL-2 production and T cell proliferation [8]. On the other hand, signals through TcR/CD3 complexes in the absence of costimulatory signals induce clonal anergy, which leads to peripheral tolerance [3,9,10]. In addition, CD152 mediates a negative role in T cell activation and is involved in the induction of peripheral tolerance [11–13]. CD80 and CD86 appear to differ in their ability to differentiate and maintain Th1 and Th2 cells [14–18]. Thus it is useful to understand the function and expression of these costimulatory molecules in allergic rhinitis.

We reported that CD86 is selectively and antigen-specifically up-regulated in peripheral blood B cells from patients [19]. Hofer *et al.* have also demonstrated CD86 up-regulation on peripheral blood B cells from patients with allergic asthma, which is another prototypic disease of immediate hypersensitivity [20]. Van Neerven *et al.* described that allergen-specific T cell proliferation and cytokine expression require CD28-CD86 costimulation [21]. In addition, we recently found that house dust mite-specific T cells generated from the peripheral blood of patients with perennial rhinitis express both CD80 and CD86 and function through T–T cell interactions [22].

In the present study, we determined the expression of CD80/CD86 molecules and their counter-receptors in the nasal mucosa, the actual inflammatory site of rhinitis, with and without intranasal allergen provocation. We compared expression of these molecules in patients with and without perennial allergic rhinitis. Furthermore, we analysed the fluctuations of these expressions between chronic allergen exposure and allergen provocation.

Patients and methods

Patients

Eighteen Japanese patients (19–42 years old, mean 26.6 ± 8.6 years, 10 males and eight females) with perennial allergic rhinitis were studied. All of them had severe symptoms graded according to the criteria outlined by Okuda *et al.* [23], exhibited positive titres of IgE to *Dermatophagoides pteronyssinus* ranging from 2.44 to >100 UA/mL when assayed by the CAP-RAST, and responded positively to the nasal provocation test using paper discs containing house dust (Torii Co., Tokyo, Japan). Sixteen Japanese patients (19–64 years old, mean 30.4 ± 17.9 years, eight males and eight females) with either chronic hypertrophic rhinitis or mucocoele of paranasal sinuses were selected as controls. None of the control subjects had a history of allergic diseases and none responded in the nasal provocation test. Informed consent for participation in the study was obtained from each subject. None of the

patients received immunotherapy or used immunosuppressive drugs including steroids during this study.

Sampling of the nasal mucosa

In eight out of 18 patients with allergic rhinitis and eight out of 16 control subjects, three paper discs containing extracts of house dust were placed onto the inferior turbinate of the nose. Twelve hours later, the turbinate samples were surgically excised. Moreover, in four out of eight subjects in each group, samples were consecutively excised at different time points. Tissue samples were embedded immediately in OCT compound (Miles Inc., Indianapolis, IN, USA), frozen in liquid nitrogen, and stored at -80°C until use. The samples were surgically excised without the nasal provocation in the remaining eight and eight of allergic and non-allergic patients, respectively.

Antibodies

Purified anti-human CD80 (L307; mouse IgG1, κ) and CD86 (FUN-1; mouse IgG1, κ) mAbs have been described [24,25]. Purified anti-human CD28 (CD28.2; mouse IgG1, κ), and CD152 (BNI 3; mouse IgG2a, κ) and PE-labelled anti-human CD86 (FUN-1) mAbs were purchased from PharMingen (San Diego, CA, USA). Purified anti-human CD3 (UNHT1 mouse IgG1, κ), CD19 (HD37, mouse IgG1, κ) and FITC-labelled anti-human CD1a (NA1/34, mouse IgG2a, κ), CD3 (UNHT1 mouse IgG1, κ), CD14 (TUK4, mouse IgG2a, κ), and CD19 (HD37, mouse IgG1, κ) were purchased from Dako (Tokyo, Japan).

Immunohistochemistry

Cryostat sections (6 μm thick) of nasal turbinate samples were air-dried and fixed in acetone for 10 min. After washing with PBS, non-specific protein staining was blocked with PBS containing 10% normal goat serum, followed by a wash with PBS containing 0.05% Tween 20 (PBS–T). Sections were incubated with purified mouse anti-human CD3, CD19, CD80, CD86, CD28, CD152, or respective isotype control mAbs for 1 h at room temperature. After washing with PBS–T, endogenous peroxidase was blocked by adding 0.3% H_2O_2 for 5 min at room temperature. After washing with PBS–T, the sections were incubated with goat anti-mouse immunoglobulins conjugated to peroxidase-labelled dextran polymer (Dako Envision+ Dako), according to the manufacturer's instructions, for 30 min at room temperature and washed with PBS followed by reaction with diaminobenzidine tetrahydrochloride (0.2 mg/ml) and 0.003% H_2O_2 . After washing with deionized distilled H_2O , the sections were counterstained with haematoxylin, dehydrated in ethanol,

and coverslips were mounted in xylene with resin. The numbers of cells bearing CD80/CD86 molecules and their counter-receptors were counted in four fields of high power (10×40), and an average was determined.

Immunofluorescence

Samples were double-stained for surface markers and CD86 using an enzyme antibody method. Cryostat sections were air-ried, fixed and blocked as described above, then the sections were incubated with a mixture of PE-labelled mouse anti-human CD86 and FITC-labelled mouse anti-human lymphocyte surface markers as follows for 1 h at room temperature: CD1a, CD3, CD14, CD19. After washing with PBS-T followed by PBS, coverslips were mounted with fluorescent mounting medium (Vector Labs, Burlingame, CA, USA). Sections were then photographed under a fluorescence microscope.

Statistical analysis

Statistical comparisons were performed using Student's unpaired *t*-tests. A level of $P < 0.05$ was considered statistically significant.

Results

Expression of CD80 and CD86 in nasal mucosa

The expression of CD80 was not different in nasal mucosa between allergic and non-allergic subjects without allergen provocation, which reflected the chronic exposure of house dust. However, the expression was significantly increased in allergic subjects 12–15 h following the nasal provocation with house dust (Fig. 1a). In non-allergic subjects no significant changes could be seen following the provocation.

On the other hand, the expression of CD86 was higher in nasal mucosa of patients with allergic rhinitis compared with those of non-allergic patients even without allergen provocation. In addition, this expression was significantly increased after the nasal provocation with house dust (Fig. 1b). Similar to the expression of CD80, the expression of CD86 was not changed following the allergen provocation in non-allergic subjects.

When we assayed the kinetics of CD80 and CD86 expression in nasal mucosa, we observed that the expression of CD80 was induced at 12 h following the nasal provocation in allergic patients. On the other hand, half of the samples showed the increased expression of CD86 at 6 h, and all the samples exhibited the peaked expression at 12 h. Similar levels of expression of both molecules were detected until 24 h. However, we observed no remarkable changes in either CD80 or CD86 expression in nasal mucosa of non-allergic patients (Fig. 2).

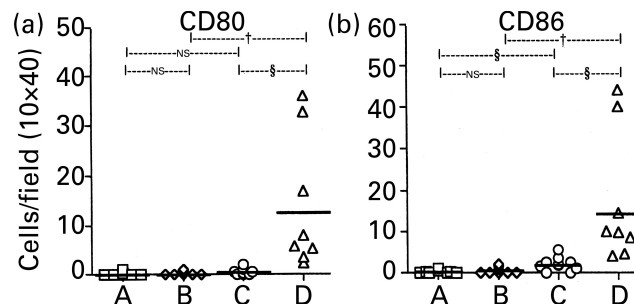


Fig. 1. Numbers of cells expressing CD80 (a) and CD86 (b) in nasal mucosa of non-allergic (A, B) and allergic (C, D) patients. Nasal turbinates were excised 12 h after nasal provocation with house dust (B, D) or without provocation (A, C), and immunohistologically stained using the Envision system. Positive cells were counted microscopically in randomized four high power fields (10×40), and the average was calculated. Bar represents mean number of positive cells. A, Non-allergic subjects without provocation ($n = 8$); B, non-allergic subjects with provocation ($n = 8$); C, allergic subjects without provocation ($n = 10$); D, allergic subjects with provocation ($n = 8$). $†P < 0.01$; $§P < 0.005$. NS, Not significant.

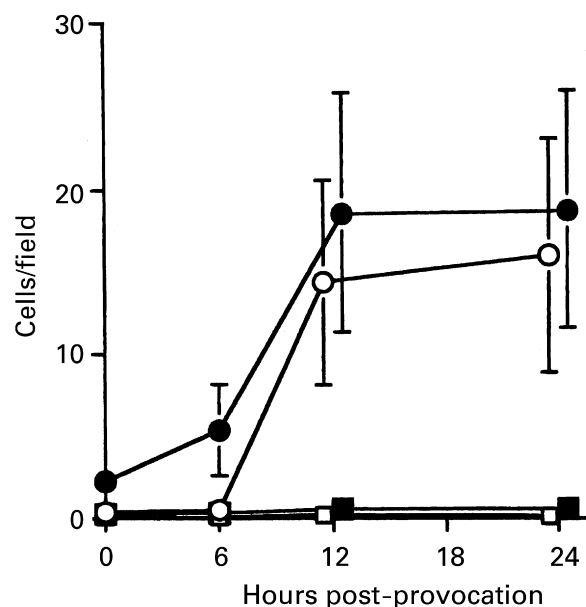


Fig. 2. Kinetics of CD80 and CD86 expression in nasal mucosa following nasal provocation with house dust in allergic ($n = 4$) and non-allergic ($n = 4$) patients. Nasal turbinates were consecutively excised at 0, 6, 12, and 24 h after nasal provocation, and positive cells expressing CD80 and CD86 were counted in randomized four high power fields (10×40), and the average was calculated. ○, CD80 on allergic patients; ●, CD86 on allergic patients; □, CD80 on non-allergic patients; ■, CD86 on non-allergic patients.

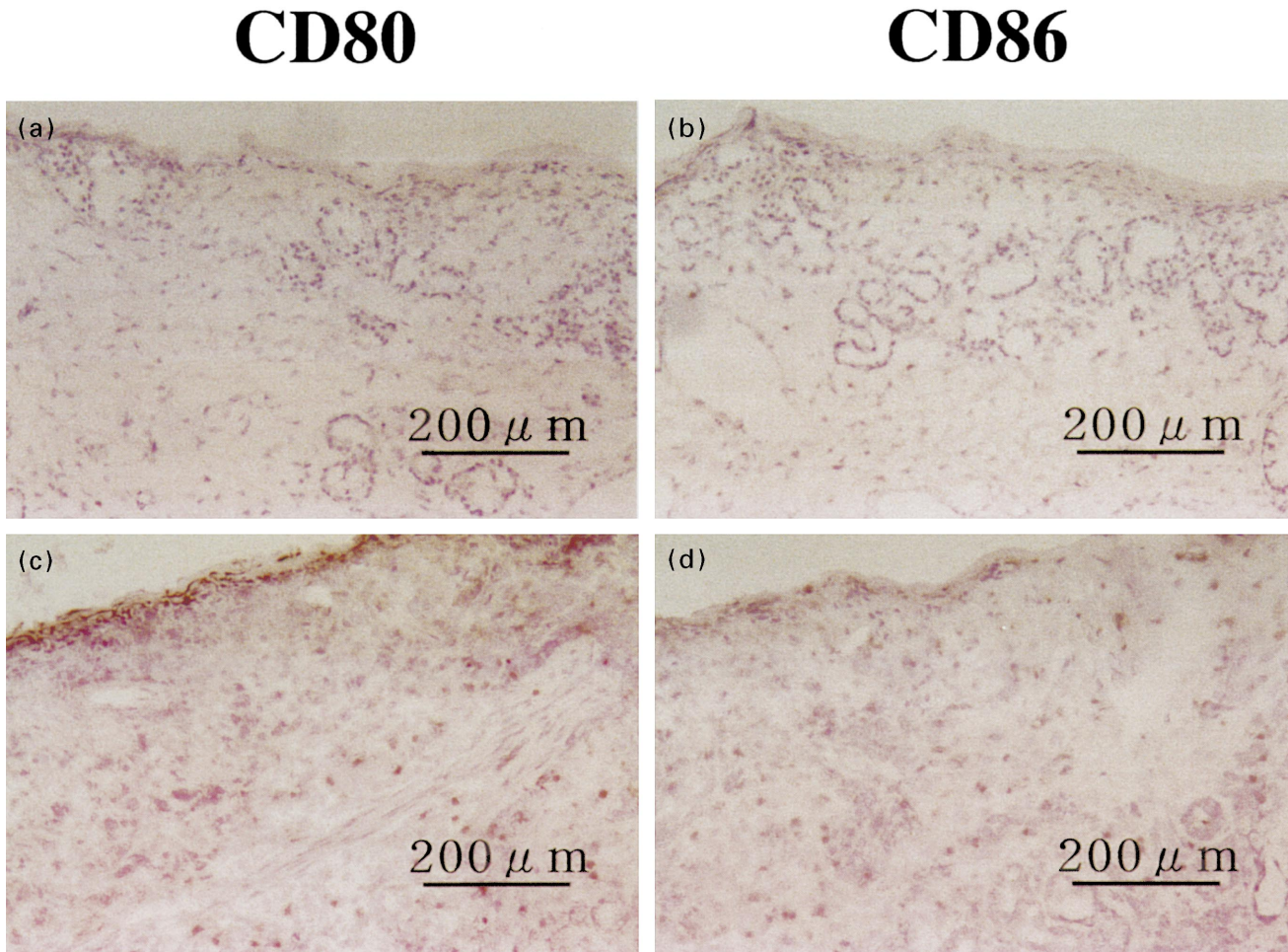


Fig. 3. Typical immunohistochemical staining of CD80 and CD86 in nasal mucosa. Three paper discs containing extracts of house dust were placed onto inferior turbinate from non-allergic patients (a,b) or patients with perennial allergic rhinitis (c,d). Twelve hours later, the turbinate samples were excised, embedded and frozen as described in Patients and Methods. Nasal tissues were stained using the Dako Envision+ system for CD80 (a,c) and CD86 (b,d), respectively. Bar = 200 μ m.

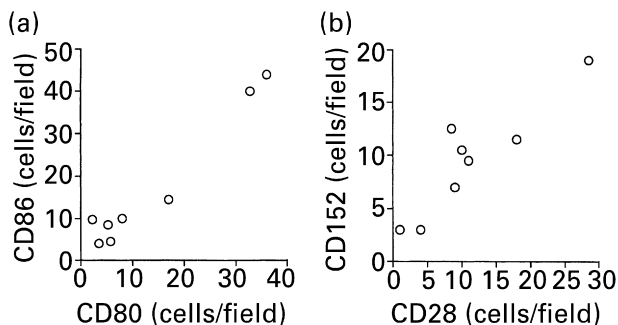


Fig. 4. Correlation of expression of costimulatory molecules in nasal mucosa. Numbers of cells positively stained for CD80, CD86, CD28, and CD152 per high power fields (10×40) 12 h after allergen provocation were analysed in eight allergic patients. (a) Correlation of CD80 and CD86 expression. (b) Correlation of CD28 and CD152 expression.

Cells expressing CD80/CD86 molecules were located mainly in the superficial layer of lamina propria followed by the epithelial layer, and the proportion of positive cells was very low in the deep layer of the lamina propria (Fig. 3). We also observed that the number of CD3⁺ and CD19⁺ lymphocytes that infiltrated the layer of the lamina propria was significantly higher in patients with allergic rhinitis compared with controls (data not shown). There was no significant difference between the number of CD80 and CD86 molecules in the tissue, but the expression was positively correlated ($r = 0.974$; Fig. 4a).

Expression of counter-receptors for CD80/CD86 in nasal turbinate

In the absence of allergen provocation, the expression of both CD28 and CD152 in nasal mucosa was similar

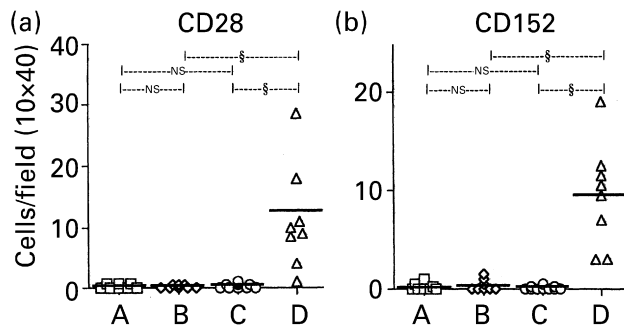


Fig. 5. Numbers of cells expressing CD28 (a) and CD152 (b) in nasal mucosa of non-allergic (A, B) and allergic (C, D) patients. Nasal turbinates were excised 12 h after nasal provocation with house dust (B, D) or without provocation (A, C), and immunohistologically stained using the Envision system. Positive cells were counted microscopically in randomized four high power fields (10×40), and the average was calculated. Bar represents mean number of positive cells. A, Non-allergic subjects without provocation ($n = 8$); B, non-allergic subjects with provocation ($n = 8$); C, allergic subjects without provocation ($n = 10$); D, allergic subjects with provocation ($n = 8$). $\$P < 0.005$. NS, Not significant.

between patients with and without allergic rhinitis. However, 12 h following nasal provocation with house dust, both molecules were induced significantly more in allergic patients compared with non-allergic controls (Fig. 5). The distribution of cells expressing either CD28 or CD152 was similar to that of cells expressing CD80 and CD86 (Fig. 6). The expression of CD28 and CD152 was positively correlated ($r = 0.906$, Fig. 4b), although the expression of CD80/CD86 and their counter-ligands was not strongly correlated (data not shown).

Phenotype analysis of cells expressing CD86 molecules

We analysed the phenotypes of nasal cells expressing CD86 by double-staining immunofluorescence. In allergic patients, most CD19⁺ cells expressed CD86 (Fig. 7a). In addition, not only CD1a⁺ and CD14⁺, but also some CD3⁺ cells expressed CD86 in nasal mucosa (Fig. 7b-d). Among the CD86-bearing cells, $35.42 \pm 6.9\%$, $11.25 \pm 1.3\%$, $7.92 \pm 2.42\%$, and $12.92 \pm 2.95\%$ were CD19⁺, CD14⁺, CD1a⁺ and CD3⁺, respectively ($n = 6$). On the other hand, $28.40 \pm 7.13\%$ of CD152-bearing cells expressed CD86 ($n = 5$).

Discussion

The present study revealed that, without nasal provocation, patients with perennial allergic rhinitis showed increased expression of CD86 but not CD80 in the nasal mucosa

compared with control subjects. In addition, following nasal provocation with house dust, expression of both CD80 and CD86 was increased in the nasal mucosa from allergic patients compared with that from control subjects. We obtained nasal turbinates 12 h after nasal provocation, which appears to reflect allergen-induced late-phase response [26]. One current argument is whether or not costimulation via CD80 and CD86 can differentially regulate Th cell differentiation into Th1 or Th2 [14–18]. The degree of expression between the two molecules was not significantly different, suggesting that both CD80 and CD86 are involved in the pathogenesis of nasal mucosa in allergic rhinitis. CD86 is believed to be constitutively expressed on dendritic cells and macrophages, and the expression is rapidly induced or enhanced on APC and activated T cells in response to antigen stimulation. On the other hand, CD80 appears more slowly and often at significantly lower levels [27]. In fact, the expression of CD80 was induced at 12 h following nasal provocation in allergic patients, whereas the expression of CD86 was induced at 6 h and peaked at 12 h. These results suggest that the expression of CD80 and CD86 in the nasal mucosa in response to *in vivo* allergen challenge shows different kinetics, and this appears to be consistent with previous *in vitro* findings [19,27]. In contrast, no remarkable change in either CD80 or CD86 expression was seen in nasal mucosa of non-allergic patients.

CD86 was expressed on CD19⁺, CD1a⁺ and CD14⁺ cells, and some CD3⁺ nasal lymphocytes also expressed CD86. CD86 is expressed on APC and activated T cells after antigen stimulation [5,15,23,28–30]. Thus, nasal T lymphocytes may also be involved in T–T cell interactions. In addition, CD80/CD86 molecules were expressed in the epithelial layer of the nasal mucosa (Fig. 3). The cells expressing CD80/CD86 molecules in the epithelial layer appeared to be Langerhans cells since they also expressed CD1a (data not shown), a finding consistent with our previous results [31,32]. Recent studies have suggested that other types of non-professional APC such as epithelial cells or keratinocytes express CD80/CD86 molecules [33,34]. The expression of CD80/CD86 on non-professional APC in nasal mucosa such as mast cells, epithelial cells, or fibroblasts should be investigated.

The current data show that the level of local CD28 expression in the nasal mucosa was significantly higher in allergic than in non-allergic individuals after allergen provocation *in vivo*. However, CD28 expression was not different between allergic and non-allergic subjects without the provocation. The relationship remains unclear. Sensitivity for the detection of the molecule might affect the results. Signal transduction through CD28 synergized with the TcR signal is important in T cell activation, which leads to IL-2 secretion and subsequent T cell differentiation

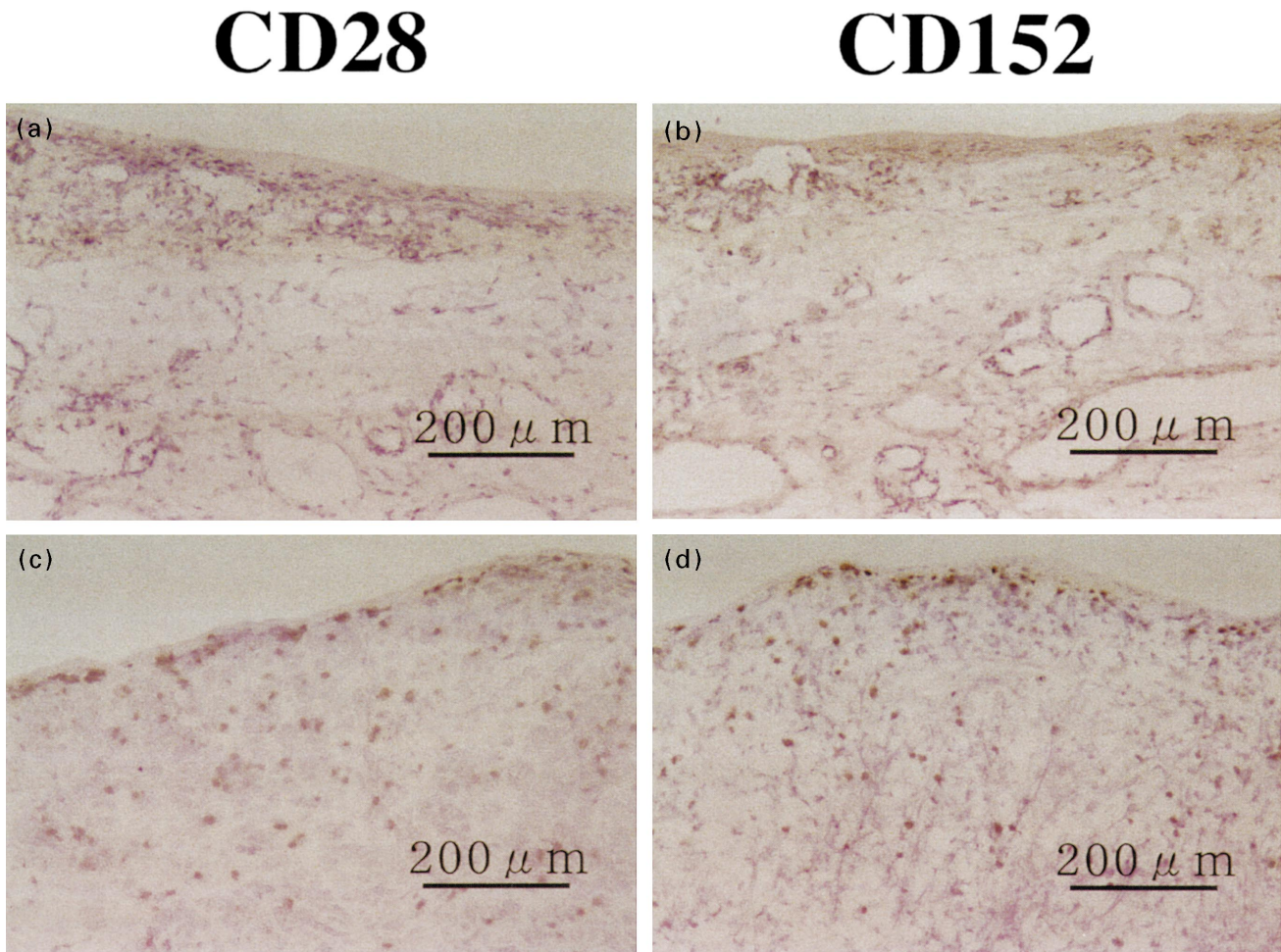


Fig. 6. Immunohistochemical study of CD28 and CD152 in nasal mucosa. Three paper discs containing extracts of house dust were placed onto inferior turbinate samples from non-allergic individuals (a,b) or patients with perennial allergic rhinitis (c,d). Twelve hours later, the turbinate samples were excised, embedded and frozen as described in Patients and Methods. Nasal tissues were stained using the Dako Envision+ system for CD28 (a,c) and CD152 (b,d), respectively. Bar = 200 μ m.

[9,35,36]. Tsicopoulos *et al.* described that CD28 is expressed at higher levels on peripheral blood T cells from patients who are allergic to bee venom than from those of control individuals [37]. The level of CD28 expression on CD4⁺CD45RO⁺ T cells is increased when peripheral blood mononuclear cells (PBMC) from patients with perennial rhinitis are cultured with mite antigen [22]. Our data appear to be consistent with these studies that used T cells from PBMC, and therefore suggest that the up-regulation of CD28 expression on T cells orchestrates the ongoing allergic response through CD80/CD86-CD28 ligation.

CD152 is not constitutively expressed on quiescent T cells, but it can be detected following TcR ligation and further augmented by CD28 costimulation [15]. Recent studies indicate that CD152 can deliver a negative signal to activated T cells [11–13]. The level of CD152 expression

was remarkable in nasal mucosa from patients with perennial rhinitis but not in that from non-allergic controls after allergen exposure (Fig. 5). This may be due to the activation of antigen-specific T cells after allergen exposure and suggests that an excessive local T cell response against inhaled antigen might be repressed through CD152 signals. However, whether or not the function of CD152 expressed in atopic patients is similar to that in non-atopic individuals remains unclear. One explanation for exacerbated nasal T cell response in allergic individuals is that the CD152 expressed in atopic patients may not deliver sufficient negative signals following antigen exposure, which fails to repress the allergic response.

In conclusion, this study revealed the selective local expression of CD80/CD86 molecules and their counter-receptors at actual sites of allergic inflammation in allergic

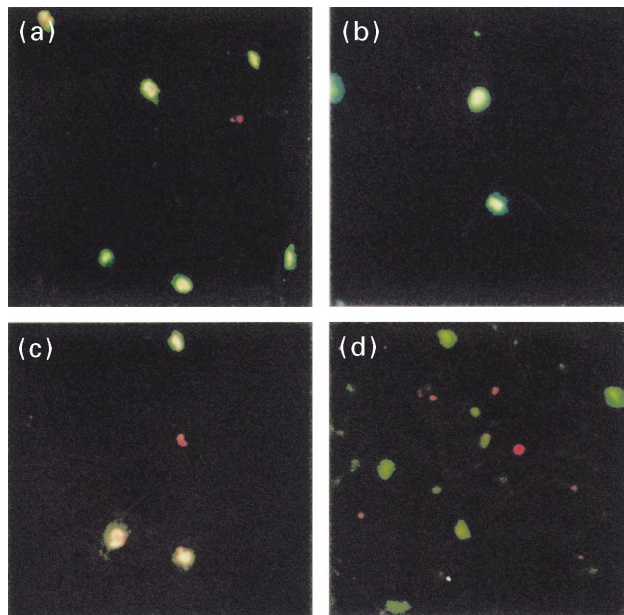


Fig. 7. Expression of CD86 in infiltrating CD19, CD1a, CD14, and CD3 lymphocytes in nasal mucosa. Samples were double-stained using immunofluorescence-labelled antibody method. CD19 (a), CD1a (b), CD14 (c) and CD3 (d) were labelled with FITC and CD86 was labelled with PE. Sections were photographed under a fluorescence microscope.

patients after allergen exposure. This report may provide a basis for the future therapeutic approaches in the management of allergic rhinitis, since local blockade of costimulation with CD80/CD86 inhibits the symptoms, nasal eosinophilia, and specific IgE production in presensitized mice [38].

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