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Enhanced expression of B7.2 (CD86) by percutaneous sensitization with house dust mite antigen

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Abstract

House dust mite antigen is a well-known allergen in the pathogenesis of atopic dermatitis (AD), a chronic relapsing inflammatory skin disease. We evaluated the AD model mice sensitized with house dust mite antigen and observed a Th2-dominant immune response. In this experiment, BALB/c mice were sensitized percutaneously with house dust mite antigen three times with 7 days interval after skin barrier disruption. A remarkable infiltration of polymorphonuclear granulocytes and monocytes in the cutis was observed in mice treated with this antigen, high serum IgE levels and IL-4 mRNA expression in local lymph node cells was also observed. $CD19^+$ B cell numbers overturned to $CD4^+$ helper T cells. In these mice, there was significant increase of B7.2 (CD86) expression on $CD19^+$ B cells. These results indicate that house dust mite antigen sensitizes BALB/c mice and skews their Th1/Th2 balance toward Th2.

Keywords: Atopic dermatitis (AD); House dust mite antigen; Th1/Th2; IgE; IL-4; IFN-γ; B7.1 (CD80); B7.2(CD86)

1. Introduction

Atopic dermatitis (AD) is clinically characterized by cutaneous reactions responsible for barrier-disrupted skin that is highly susceptible to hapten or antigen penetration. House dust mite antigen is a major allergen for human diagnostic and experimental use such as patch test [1] and nasal hyperresponsiveness [2]. Patients with AD, have a significant deficiency of ceramides, a major constituent of intercellular lipids in the stratum corneum [3], and ceramides provide a cutaneous permeability barrier against environmental allergens or irritants, thus probably make the skin susceptible to hapten or antigen penetration. Allergen penetration causes several reactions with Th2-dominant immunity including IL-4 and -5 production [4,5], elevated IgE synthesis [6–8] and skin infiltration of polymorphonuclear granulocytes and monocytes.

$CD4^+$ helper T cells can be subdivided into Th1 and Th2 cells, their balance called Th1/Th2 balance has been shown to be critically important in various immune responses and is essential to keep their homeostasis [9,10]. Th2 cells produce IL-4, -5 and -10 and play a key role in humoral immunity [11,12], whereas Th1 cells produce IFN-γ and IL-2 and are essential for the induction of cellular immunity [13,14]. Currently, it is controversely discussed why immune response to allergens is polarized to Th2-dominance, since no direct evidence exists showing that allergens behave as a primary factor for Th2-dominant immune response.

Interaction between T and B cell plays a pivotal role for the characteristic Ig production in AD [15]. Activation of resting lymphocytes requires at least two signals; the engagement of TCR with MHC plus a peptide complex expressed on B cells, as well as interaction between CD28/CTLA-4 on T cells with B7 on APCs

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were discussed as well-characterized as costimulatory pathway [16–18]. Two members of the B7 family, B7.1 (CD80) and B7.2 (CD86) on $CD19^+$ B cells were identified, and they have a different activity inducing Th1 versus Th2 immune response, respectively [19]. Several studies have demonstrated distinct kinetics and interaction sites between B7.1/B7.2 and CD28/CTLA-4 receptor–counter ligand system [20–23]. Such differential binding may have unique signaling properties that affect T cell activation and subsequent Th1/Th2 balance. It was suggested that generation of Th2 cells depends mainly on interaction between CD28 with B7.2 and enhanced expression of B7.2 was observed in-patients with AD.

We established the murine AD model in which house dust mite antigen-treated mice showed epidermal inflammation. This model showed typical symptoms in AD patients, histological features of lesional skin and high serum IgE level. In AD, their cellular mechanism is not fully elicited, though it is considered that Th2-dominant immune response may regulate the allergic disease. In this thesis, therefore, we analyzed IL-4 and IFN-γ mRNA expression in local lymph node cells, phenotypic analysis of T and B cell, and the expression of B7.1 versus B7.2 on B cells. There was a significant increase of B7.2 (CD86) expression on $CD19^+$ B cells with the treatment of house dust mite antigen. These results indicate that house dust mite antigen skews their Th1/Th2 balance towards Th2.

2. Materials and methods

2.1. Materials

2.1.1. Mouse

Female BALB/c mice, 5–8 weeks old were obtained from SLC Japan (Hamamatsu, Japan) and bred in specific pathogen free environment. Five to ten animals were used in each experimental group.

2.1.2. House dust mite antigen

House dust mite antigen was purchased from Torii & Co., Ltd. (Tokyo, Japan), which is a 50% glycerol saline extract of *Dermatophagoides farinae*. Originally (1:10) used for clinical diagnosis of house dust mite allergy [24]. The extract was dialyzed against PBS to remove glycerol and was concentrated to 10-fold (1:1 solution were made).

2.1.3. Antibody

All antibodies were purchased from BD PharMingen (San Diego, CA).

2.2. Induction of cutaneous inflammation

Female BALB/c mice were barrier-disrupted by repeated applications (eight times) of adhesive cellophane tape to shaved abdominal skin. Percutaneous sensitization with antigen was performed by topical application of 50 l house dust mite antigen solution (1:1) onto the barrier-disrupted abdominal skin. Same amount of vehicle, phosphate buffer saline was applied to PBS-treated mice. Each mouse was immunized three times with 7 days interval then 4 days after the third immunization, mice were sacrificed, serum, lymph node cells and abdominal skin from treated region were collected.

2.3. Histological analysis of cellular mechanisms

The treated regions of abdominal skin were collected and fixed with 10% neutral buffered formalin and embedded in paraffin. These tissue sections were stained with hemotoxylin and eosin.

2.4. Detection of serum IgE

Serum IgE level was measured by sandwich ELISA. Briefly, plates were coated with anti-mouse IgE (R35-72) in sodium carbonate buffer (100 l per well) incubated at 4 °C for 1 h. After washing three times with 0.05% Tween-20 in PBS, plates were blocked with 1% milk in PBS (300 l per well) at 37 °C for 1 h. Then final volume 50 l per well of serum samples were added and diluted with 10% FBS in RPMI1640 medium, samples were incubated at 37 °C for 1 h. Plates were washed and incubated with 100 l per well Biotin anti-mouse IgE (R35-92) antibody in Tween-20 PBS at 37 °C for 1 h. Plates were incubated with Elite ABC KIT (VECTOR Lab. Inc.; Burlingame, CA) in 1% milk in PBS (50 l per well) at 37 °C for 1 h. Finally, IgE levels were detected using TMB peroxidase substrate (KPL; Guildford, UK) and absorbance was read at 450 nm within 10 min using an ELISA reader (BIO-RAD).

2.5. Cellular cytokine mRNA expression

Cellular total RNA was prepared from inguinal and axillar lymph node cells using TRIzol reagent (GibcoBRL; Grand Island, NY). One microgram of total RNA was reverse transcripted using a Not I-d $(T)_{18}$ primer and First-Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech; Piscataway, NJ). For PCR, total of 30 cycles were done; each cycle included denaturation for 2 min at 94 °C, re-annealing for 2 min at 60 °C and extension for 1.5 min at 72. PCR primers used were summarized below. IL-4 (5′-AGT TGT CAT CCT GCT CTT CTT TCT C) and (3′-CGA GTA ATC CAT TTG CAT GAT GCT C). IFN-γ (5′-

GCT ACA CAC TGC ATC TTG GCT TTG) and (3′-CAC TCG GAT GAG CTC ATT GAA TGC). β-Actin were purchased from CLONTECH (Palo Alto, CA).

2.6. Flow cytometry

Single cell suspensions from inguinal and axiliar lymph nodes were prepared and flow cytometry analysis of cell surface phenotypes was performed as previously described [23]. Lymph node cells (5×10^5) were harvested and incubated with antibodies for 30 min at 4 °C and washed twice with PBS. PE anti-mouse CD19 (1D3), FITC anti-mouse CD3e (CD3e chain) (145-2C11), FTTC anti-mouse CD4 (L3T4), PE anti-mouse CD8 (Ly-2), FITC anti-mouse CD80 (B7.1) (16-10A1) and FITC anti-mouse CD86 (B7.2) (GL1) were used. Cell surface phenotype analysis was conducted by EPICS ELITE Cytometer (Coulter) with EPICS ELITE Flow cytometry Workstation Version 4.02. For each sample, data from 10 000 viable cells were analyzed.

2.7. Statistical analysis

Student's t-test was applied to calculate the differences between mouse groups on cell surface phenotype and protein level using STATVIEW Version 5 application. P values less than 0.005 were considered to be statistically significant.

3. Results

3.1. Histological analysis

A significant inflammation was evoked in house dust mite antigen-treated mice. Stratum corneum was detached by repealed barrier-disruption, caused hypertrophic change of stratum granulosum and prickle call layer was observed (Fig. 1). Infiltration of polymorphonuclear granulocytes and monocytes was detected in the reticular dermis and periadnexa dermis beside the hair follicles and eccrine glands. PBS-treated cutis showed slight invasion of polymorphonuclear granulocytes and monocytes to epidermis and subcutaneous tissue though comparable to non-treated cutis.

3.2. Serum IgE level in house dust mite antigen-treated mice

Serum IgE level in house dust mite antigen-treated mice was increased significantly against PBS-treated ($P < 0.001$) and non-treated mice ($P < 0.007$). PBS-treated mice showed low serum IgE level similar to non-treated mice. These data indicate that house dust mite antigen-treatment could facilitate the induction of IgE production (Fig. 2).

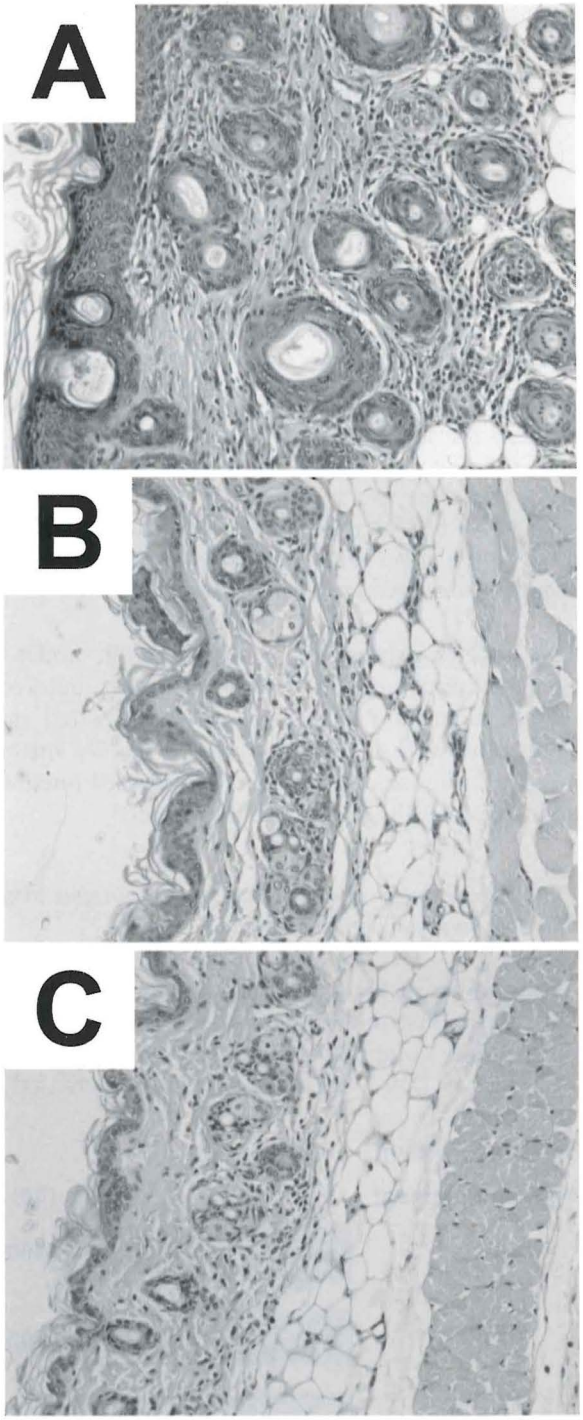

Fig. 1. Histological analysis. (A) a significant inflammation was evoked in house dust mite antigen-treated cutis, stratum corneum was detached, hypertrophy and exocytosis were observed in stratum granulosum and prickle cell layer. Infiltration of polymorphonuclear granulocytes and monocytes was detected in the reticular dermis and periadnexa dermis beside the hair follicles and eccrine glands. (B) PBS-treated cutis showed slight invasion of monocytes to epidermis and subcutaneous tissue though comparable to (C) non-treated cutis.

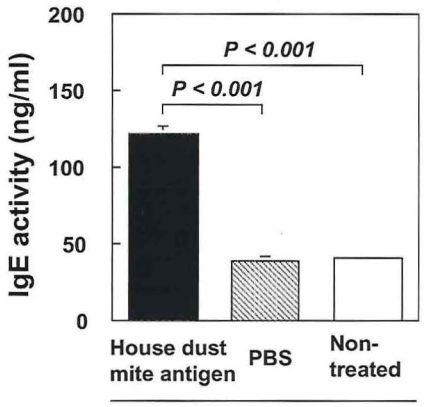

Fig. 2. House dust mite antigen treatment induced serum IgE. Serum IgE level was measured by sandwich ELISA (see Section 2). In house dust mite antigen-treated mice, serum IgE levels were increased significantly compared with PBS treated and non-treated mice. PBS-treated mice showed low serum IgE levels like non-treated mice. These data indicate that house dust mite antigen-treatment facilitates the induction of IgE production in BALB/c mice.

3.3. Cytokine mRNA expressions in the axillar and inguinal lymph nodes after sensitization with house dust mite antigen through barrier disrupted skin

IL-4 mRNA expressions in local lymph nodes of house dust mite antigen-treated mice was markedly increased, whereas PBS-treated and non-treated mice did not express IL-4 mRNA (Fig. 3). IFN-γ mRNA expression in house dust mite antigen-treated mice was comparable to PBS-treated and non-treated.

3.4. Statistical analysis of cell surface phenotypes in the axillar and inguinal lymph node cells

Total number of the axillar and inguinal lymph node cells in house dust mite antigen-treated mice increased about 1.6-fold compared with PBS-treated and non-treated mice. $CD19^+$ B cells particularly increased in

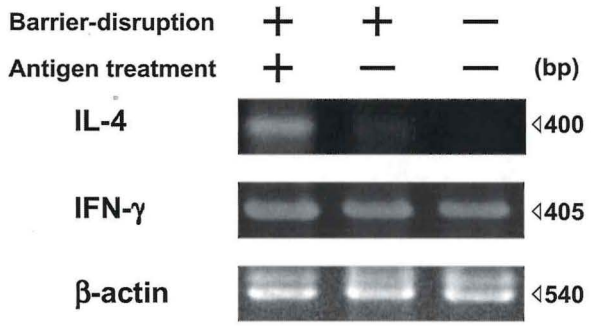

Fig. 3. Cytokine mRNA expression in the axillar and inguinal lymph node cells after sensitized with house dust mite antigen. RT-PCR was performed to detect IL-4 and IFN-γ mRNA (see Section 2). IL-4 mRNA expression of the lymph nodes in the house dust mite antigen was increased, whereas PBS-treated and non-treated mice did not express IL-4 mRNA. IFN-γ mRNA was expressed comparably in all mice. β-actin was used as positive control.

antigen-treated mice, whereas $CD4^+$ and $CD8^+$ T cell numbers in house dust mite antigen-treated mice slightly increased, but not significantly (Fig. 4). Interestingly, $CD19^+$ B cell numbers overturned to $CD4^+$ helper T cells in house dust mite antigen-treated mice.

3.5. B7 molecular expression on $CD19^+$ B cells in local lymph node cells

B7.2 molecular expression on $CD19^+$ B cells was increased in house dust mite antigen-treated mice ($74.7 \pm 1.48\%$) compared with PBS-treated ($56.0 \pm 5.39\%$; $P < 0.002$) and non-treated mice ($51.7 \pm 5.39\%$). There was no significant change of B7.1 expressions (Fig. 5). (House dust mite antigen $34.8 \pm 4.43\%$; PBS $32.3 \pm 2.85\%$; non-treated $27.2 \pm 1.55\%$, respectively). This tendency was obtained from three different experiments.

4. Discussion

In AD patient, the skin barrier is disrupted and allows a variety of antigens, such as viruses, fungi and so on, to penetrate into the dermis [25]. There is a significant deficiency in ceramides, a major constituent of intercellular lipid in the stratum corneum, in both lesional and non-lesional skin, thus probably making the skin susceptible to hapten or macromolecular antigen penetration. Several studies demonstrated that dry skin in AD is caused by transepidermal water loss, reduction of ceramide and disorders of enzymes. It is also known that lipid in stratum corneum works as a water retainer [26] as well as permeability barrier [27] by forming a multilamella structure in the stratum corneum. Some reports suggested that AD is mainly associated with the diminished water-permeability and deficient water holding properties. The relationship between barrier dysfunction, type 1 allergy, type IV allergy and T cell disorder is quite complex [3,24,28]. Therefore, the establishment of mouse AD model is definitely of great importance for the elucidation of the pathogenesis of the disease and in developing new approaches for therapy [29].

To induce cutaneous inflammation in a mouse model, BALB/c were sensitized with a high-molecular-weight protein antigen, house dust mite antigen through barrier-disrupted skin. Previously, OVA and haptens such as tri-nitrochloro-benzene (TNCB) or piclyl chloride (PiCl) were used as allergen to induce anaphylaxis response, though these were not common allergens for human AD. In human, house dust mite antigen (saline extract of *D. farinase*) used in our experiments is employed for clinical diagnosis of house dust mite allergy. That is considered to be the most common allergenic pathogenesis for AD and recently this antigen

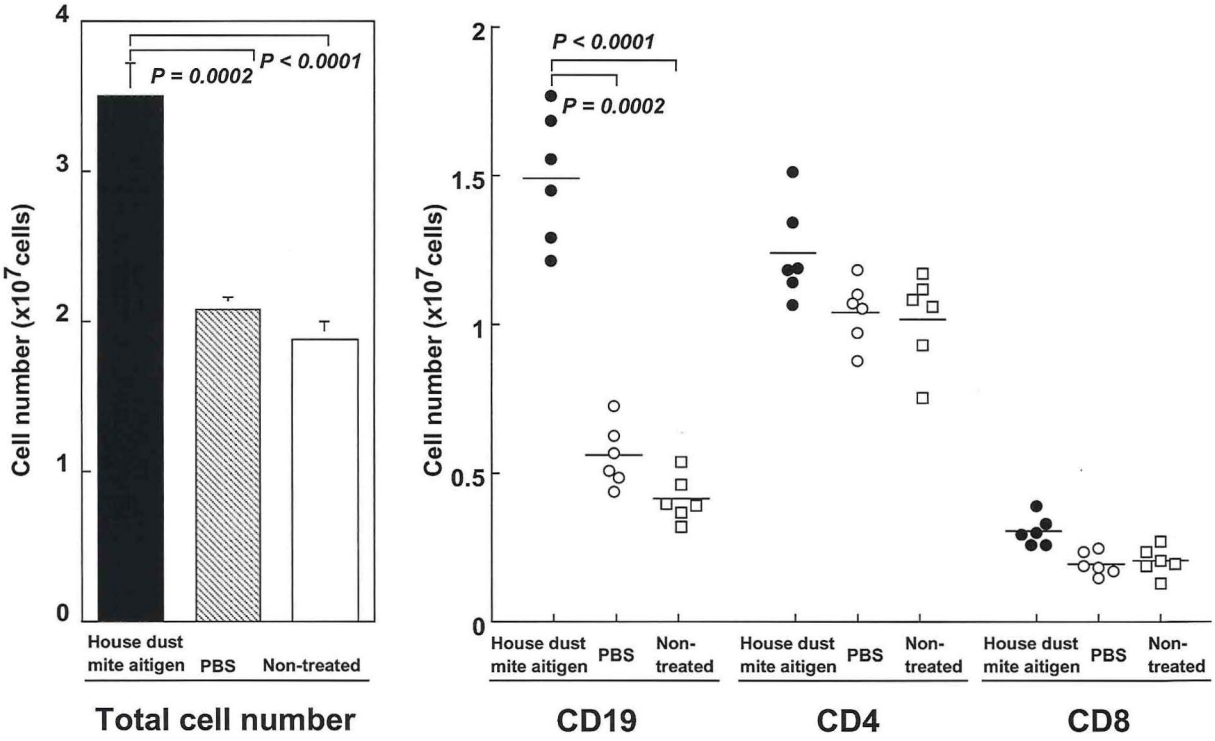

Fig. 4. Cell surface phenotypes in the axillar and inguinal lymph node cells. Total cell number of the lymph nodes was increased about 1.6-fold and CD19$^+$ B cells were particularly increased in house dust mile antigen-treated mice compared with PBS-treated and non-treated mice. CD4$^+$ and CD8$^+$ T cells were slightly increased, but it was not significantly. Interestingly, CD19$^+$ B cell numbers were overturned to CD4$^+$ helper T cells in house dust mite antigen-treated mice.

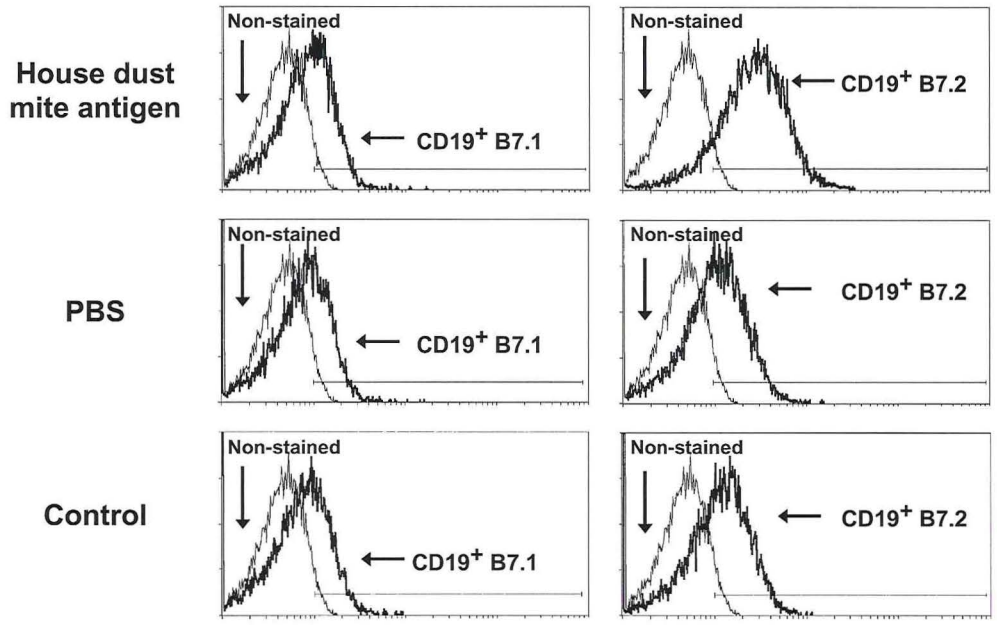

Fig. 5. B7.2 (CD86) expression was enhanced in BALB/c mice sensitized with house dust mite antigen. Axillar and inguinal lymph node cells were stained with PE anti-CD19 and FITC anti-B7.1 or FITC anti-B7.2 and analyzed with EPICS ELITE cytometer. These histograms were compared with non-stained (without fluorescence-Ig) lymph node cells. B7.2 expression on CD19$^+$ B cells was increased in house dust mite antigen-treated mice (74.7$\pm$1.48%) compared with that in PBS-treated (56.0$\pm$5.39% $P < 0.002$) and non-treated mice (51.7$\pm$5.39%). There was no significant change of B7.1 expression (House dust mite antigen 34.8$\pm$4.43%; PBS 32.3$\pm$2.85%; non-treated 27.2$\pm$1.55%, respectively).

is experimentally used [30–32]. Quite recently, Matui et al., proposed the evidence Th2-prone dermatitis using very similar percutaneous sensitization protocol, [33] in which, mice were barrier-disrupted and sensitized with house dust mite antigen, then after 7 days mice was challenged with house dust mite antigen or lipoteichoic acid (LTA). In those case there is no IL-4 and IL-5 mRNA expression after the primary sensitization, though after 7 days challenge IL-4 and IL-5 mRNA were clearly expressed in local cutis. We also examined three times immunization with 4 days interval protocol that indicated similar but weak results (data not shown). Therefore, at any rate, we thought it is negligible that the influence of mechanical skin barrier-disruption in this mouse AD model.

In our model, a significant inflammation was evoked in house dust mite antigen-treated cutis with hypertrophy and exocytosis in stratum granulosum and pickle cell layer. Infiltration of polymorphonuclear granulocytes and monocytes were detected in the reticular dermis and periadnexa dermis beside the hair follicles and eccrine glands. Serum IgE level in house dust mite antigen-treated mice increased significantly compared with PBS-treated and non-treated mice. These data indicated that house dust mite antigen treatment facilitated the induction of IgE production in mice. Bischoff et al., reported that IgE combined to Fcε receptors on the surface of mast cells triggered IgE receptor cross-linking and consequent mediator releases [34]. This manner may be a genetic trait; a high-affinity receptor for IgE shown to be mutated in AD patients [35]. Hershey et al., also reported the association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor [36].

However, it remains unclear what cellular mechanisms are operative in enhancing the emergence of Th2 responses to the treatment of house dust mite antigen. In our experiment, IL-4 mRNA expression of the inflammatory regional lymph nodes was elevated with the treatment of house dust mite antigen. On the other hand, there was no significant change in IFN-γ mRNA expression in lymph nodes. We observed that IL-4 which is secreted from Vβ 8.2* $CD4^+$ $CD62L^-$ $CD45RB^-$ T cells predominantly skewed Th cells into Th2 in the early phase of the differentiation period in BALB/c mice [37]. Several reports have suggested proinflammatory activities of IL-4. Infiltration of monocytes has been observed in IL-4 transgenic mice [38]. Polyclonal Th2 cells have been shown to mediate IL-4-dependent tissue inflammation [39]. Our study provides an interesting implication for immunological aspects of atopic disease. Many studies have recently indicated that Th2 immune responses are dominant against a specific antigen in AD [5,40].

Differentiation of naive $CD4^+$ T cells into cytokine-secreting effective Th1/Th2 cells is influenced by antigen dose, the affinity of antigen for TCR and so on. The primary response of naive $CD4^+$ T cells derived from OVA-specific TCR-transgenic mice (OVA23-3) upon stimulation with varying doses of the antigenic peptide, IL-4 expression was maximal with 50 nM Ag and this IL-4 expression was decreased significantly with increasing doses of Ag [41]. The strength of TCR signaling from MHC controls the involvement of CD28 costimulation in selective $CD4^+$ T cell differentiation. Naive $CD4^+$ T cells are receptive to CD28-dependent IL-4 production only if they receive a weak TCR signal [42,43]. During T cell-dependent humoral immune responses. Th cells provide critical costimulatory signals to B lymphocytes [44]. Although the mechanism controlling activation of naive B cells is not fully elucidated, it is known that cognate interaction between T and B cells is important in immunoglobulin production [45]. In our experiment, total cell number of the lymph nodes increased about 1.6-fold. Particularly, $CD19^+$ B cells increased in house dust mite antigen-treated mice, though $CD4^+$ T cells and $CD8^+$ T cells did not show significant change in all mice. Interestingly, $CD19^+$ B cell numbers were overturned to $CD4^+$ helper T cells in house dust mite antigen-treated mice. These data strongly suggest that increased number of B cells and IL-4 production from T cells may play a pivotal role for IgE production and Th2 response with house dust mite antigen treatment.

We investigated another cell surface molecule B7.1 and B7.2 on B cells that interacts with CD28/CTLA-4 on T cells. A role for CD28/B7 interaction in IgE synthesis has been suggested. Jirapongsananuruk et al., reported the relationship between B7.2 expression and IgE synthesis in AD patient [19]. Kuchoo et al. demonstrated the B7.1 and B7.2 costimulatory molecules differentially activate Th1/Th2 developing pathway [46]. Nakada et al., have reported that selective upregulation of CD86 on B cells by a challenging antigen play a critical role in the development of Th2 cells in peripheral blood mononuclear cells from atopic patient [23]. Previously, requirement of CD28–CD86 costimulation for allergen specific T cell proliferation and cytokine expression including IL-4 mRNA were reported using house dust mite antigen in asthma patient [47]. Repetitive costimulation of $CD4^+$ $CD45RA^-$ T cells with B7.2 results in moderate levels of both IL-4, IL-2 and towards Th0/Th2, whereas repetitive costimulation with B7.1 results in high levels of IL-2 and low levels of IL-4 [48]. These reports partially support our hypothesis. Our data suggested B7.2 expression on $CD19^+$ B cells was increased in house dust mite antigen-treated mice compared with PBS-treated and non-treated mice, though B7.1 on $CD19^+$ B cells were comparable in all mice. Tsuyuki et al., have been reported that CTLA-4 Ig treatment to abolish the interaction with CD28 suppressed the

production of IL-4, IL-5 and IL-10 from lung T cells. Consequently anti-B7.2 mAb markedly suppressed the development of IL-4 and IL-10-producing cells, IgE production and airway hyperresponsiveness, whereas blockage of B7.1 facilitated differentiation towards IL-4 production and inhibited IFN-γ secretion, and hyperresponsiveness was not blocked [49]. Taken together, these data suggest that increased B cell expression of B7.2 is not specific to AD but is observed in other human allergic diseases as well. Though there is no evidence that the B7.2 molecules on $CD19^+$ B cells plays the pivotal role in $CD4^+$ T cells differentiate into Th2 dominant using mouse AD model previously. Our experimental model, house dust mite antigen-treated BALB/c mice showing significant increase of B7.2 (CD86) expression on $CD19^+$ B cells, was strongly supported by these previous studies.

Based on these observations, we propose the following model; BALB/c mice were sensitized with house dust mite antigen using the tape-stripping method, their cellular profile indicated Th2-dominance. As an animal model system, the percutaneous sensitization protocol described here has a clear advantage over conventional immunization protocols where adjuvants are indispensable for sensitization with high-molecular-weight antigens. It is considered that AD is characterized by cutaneous reaction resulting barrier-disrupted skin, the skin being susceptible to hapten or antigen penetration. In inflammatory sites, cellular infiltration into the cutis was observed and serum IgE level was increased. The cellular mechanism to drive AD is thought to be as below; T cells are activated by cognate (primary) interaction through TCR with antigen and noncognate interactions (costimulatory signals) from B7 molecular on APCs to CD28 on $CD4^+$ T cells, resulting in IL-4 secretion leads to Th2-dominant cell responses. Furthermore, interaction with B7.2 expressed on B cells stimulated the IgE synthesis and high serum IgE level was detected as a result. This murine cutaneous inflammation model provides the first step to understand the pathogenesis of atopic diseases.

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