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Original Article

The optimal age for epicutaneous sensitization following tape-stripping in BALB/c mice

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Abbreviations:

DCs	dendritic cells
LCs	Langerhans cells
OVA	ovalbumin
qPCR	quantitative PCR
FITC	fluorescein isothiocyanate

ABSTRACT

Background: Direct contact of food proteins with eczematous lesions is thought to be the main cause of epicutaneous sensitization. To further investigate the development and pathogenesis of food allergy *in vivo*, a good mouse model of epicutaneous sensitization is needed. However, a fundamental problem in that regard is that the optimal age for epicutaneous sensitization of mice is unknown. In this study, we attempted to elucidate that optimal age.

Methods: Dorsal skin of wild-type BALB/c female mice (1, 3, 8 and 24 weeks old) was shaved, depilated and tape-stripped. A Finn chamber containing a 20-μl-aliquot of 20-mg/ml (OVA) was applied to the tape-stripped skin on 3 consecutive days/week, for 3 weeks. The body temperature was measured after intraperitoneal OVA challenge. Serum OVA-specific IgE titers and OVA-induced cytokine production by spleen cells were measured by ELISA. Dendritic cells (DCs) that migrated to the draining lymph nodes were quantified by FITC-labeled OVA and flow cytometry. The mRNA expression levels in the dorsal skin were measured by qPCR.

Results: A significant age-dependent body temperature decline was observed after OVA challenge. The serum OVA-specific IgE titer, OVA-induced cytokine production (i.e., IL-4, IL-5 and IL-13) by spleen cells, and number of FITC-OVA-engulfing DCs increased with age. In addition, mRNA for IL-33, but not TSLP or IL-25, was significantly induced in the skin by tape-stripping and increased with age.

Conclusions: Twenty-four-week-old mice showed the greatest DC migration, Th2 polarization, IgE production and body temperature decline. Skin-derived IL-33 is likely to play key roles in those changes.

Introduction

The prevalence of food allergy has increased in recent decades all over the world, especially in industrialized countries.[1] Because of that increased prevalence and occasional fatal anaphylaxis, food allergy has become a major health burden in children.[2] Almost 99% of food allergies are mediated by antigen-specific IgE antibodies; thus, IgE sensitization is a critical event in the development and pathogenesis of food allergies.

Recent studies have shown that atopic dermatitis is a robust risk for IgE-mediated food allergies.[3] In a mouse model, exposure of intact skin to antigens induced tolerance, whereas exposure of damaged skin to antigens induced sensitization,[4] suggesting that direct contact of eczematous lesions with food proteins is likely to be the main mechanism for epicutaneous sensitization.[5]

A good mouse model of epicutaneous sensitization is needed in order to further investigate the pathogenesis of food allergy *in vivo*.[6] It was reported that several factors, including the animal strain, microbiome, age and others, are involved in IgE sensitization in general.[7] With respect to age, mouse skin in early infancy (13 days old) is reportedly rich in regulatory T cells and tends to tolerate

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skin commensal microbes,[8] whereas older mice (18 months old) showed impaired production of IgE to epicutaneously exposed proteins.[9] However, the optimal age for epicutaneous sensitization in a mouse model is unknown. Clarifying the optimal age and the underlying mechanisms would be useful for establishing a better murine model of epicutaneous sensitization and that information may provide some hints for future intervention strategies.

In this study, we attempted to elucidate the optimal age for the mice for induction of food allergy through epicutaneous sensitization, and the mechanisms behind this sensitization.

Methods

Mice

Wild-type BALB/c female mice at 1, 3, 8 and 24 weeks of age were purchased from SLC Japan (Shizuoka, Japan). They were housed under specific-pathogen-free conditions at the National Research Institute for Child Health and Development. The animal protocols were approved by the Institutional Review Boards of the National Research Institute for Child Health and Development and the Institute of Medical Science, The University of Tokyo.

Epicutaneous sensitization

Epicutaneous sensitization to ovalbumin (OVA) was performed based on a previous study,[10] with some modification. In brief, the dorsal skin of mice was shaved with electric clippers, depilated using a depilatory cream (Veet®; Reckitt Benckiser, Slough, Berkshire, UK), and then tape-stripped using a transparent film dressing (Cellotape; Nichiban, Tokyo, Japan) on the same day. After that, a 20-μl-aliquot of 20-mg/ml OVA (grade V; Sigma–Aldrich, St. Louis, MO, USA) in a Finn Chamber (Finn Chamber disk; 8-mm diameter; Smart Practice, Phoenix, AZ, USA) was applied to the tape-stripped dorsal skin for 3 consecutive days/week for 3 weeks.

Measurement of body temperature

Five days after the final epicutaneous exposure, each mouse was intraperitoneally challenged with OVA (1 mg/ml solution in sterile saline) at 10 μg/g mouse body weight. The rectal temperature of each mouse was measured using a digital thermometer (TD-300®; Shibaura, Saitama, Japan) at 0, 10, 20, 30, 40, 50 and 60 min after OVA challenge.

Measurement of OVA-specific IgE titers in sera

One day before intraperitoneal OVA challenge, a serum sample was collected from each mouse. The OVA-specific IgE titer in each was measured by ELISA, as described elsewhere.[11] HRP (horseradish peroxidase)-conjugated anti-mouse IgE antibody was obtained from Bethyl Laboratories (Montgomery, TX, USA).

OVA-induced cytokine production by spleen cells

OVA-induced cytokine production by spleen cells was measured as described elsewhere, with slight modification.[12] In brief, the spleen was harvested from each mouse 2 days after OVA challenge, and a spleen cell suspension was filtered through a 70-μm cell strainer (Falcon®; Corning, NY, USA), followed by erythrocyte lysis with a lysing buffer (Red blood cell lysing buffer®; Sigma–Aldrich) for 5 min at 37 °C. The spleen cells were re-suspended in RPMI1640 medium (Nacalai Tesque, Kyoto, Japan) supplemented with 10% FCS (fetal calf serum: Biological Industries; Beit Haemek, Israel) and cultured with and without OVA (200 μg/ml) for 72 h. The supernatants were harvested, and the cytokine concentrations (IL-4, IL-5, IL-13 and IFNγ) in them were measured by ELISA (eBioscience, Thermo Fisher Scientific, Kanagawa, Japan).

Quantification of dendritic cell (DC) and Langerhans cell (LC) migration to draining lymph nodes

Dorsal skin of each mouse was shaved, depilated and tape-stripped. After these procedures, each mouse was exposed to 400 μg fluorescein isothiocyanate (FITC)-conjugated OVA[13] in a Finn Chamber on the tape-stripped dorsal skin for 24 h. After FITC treatment, axillary and inguinal lymph nodes were collected and single cell suspensions were incubated with anti-mouse CD16/CD32 mAb (clone 93; eBioscience). Cells were incubated with Brilliant violet 510-conjugated anti-mouse CD45 mAbs (clone 30-F11; Biolegend, San Diego, CA, USA), APC (allophecocyanin)-conjugated anti-mouse CD11c mAbs (clone N418; Biolegend), PE (phycoerythrin)-cy7-conjugated anti-mouse MHC class II proteins IA/IE (clone M5/114.15.2; Biolegend), PE (phycoerythrin)-conjugated anti-CD207 mAb (clone 4C7; Biolegend) and Dye eFluor 780 (Affymetrix, Thermo Fisher Scientific, Kanagawa, Japan). FITC-positive dendritic cells (DCs) in the draining lymph nodes were quantified by flow cytometry, and the number of FITC-positive DCs/1,000,000 cells was shown. DCs and LCs were determined as $CD45^{+}CD11c^{+}$MHC class II $(IA/IE)^{high}$ $CD207^{-}$ and $CD45^{+}CD11c^{+}$MHC class II $(IA/IE)^{high}$ $CD207^{+}$ cells, respectively.

Quantitative measurement of mRNA expression and histological examination of the dorsal skin

Dorsal skin of mice was harvested before shaving, after shaving and depilation, and 4 h after tape-stripping. Total RNA was extracted from the dorsal skin samples using an RNeasy kit (Qiagen, Valencia, CA, USA).[14] Quantitative PCR (qPCR) for cytokine mRNA expression was performed as described elsewhere.[15] The mRNA expression levels were normalized to the GAPDH level in each sample. Before and after 3-times tape-stripping, the epidermis was stained with hematoxylin–eosin, and its thickness was measured using a light microscope (IX70; Olympus, Tokyo, Japan) equipped with a digital camera (DP11; Olympus).

Statistics

The data are shown as the median and the ranges unless otherwise noted. Differences between groups were examined for statistical significance by the Kruskal–Wallis test followed by Dunn's multiple comparisons test. Correlations between groups were examined by Spearman's rank correlation test. Statistical evaluations were performed using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA), and $P < 0.05$ was regarded as significant.

Results

Successful epicutaneous sensitization to OVA in one-week-old mice

Dorsal skin of one-week-old mice was exposed to OVA after shaving, depilating and tape-stripping (Fig. 1A). Mice whose skin had been tape-stripped three times before exposure to OVA showed a significant body temperature decline after intraperitoneal OVA challenge (Fig. 1B). However, mice tape-stripped only one time before exposure to OVA showed only a modest body temperature decline. Those 2 tape-stripping groups showed almost comparable serum OVA-specific IgE titers, both of which were significantly higher than in the control mice (Fig. 1C).

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