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Induction of atopic eczema/dermatitis syndrome-like skin lesions by repeated topical application of a crude extract of *Dermatophagoides pteronyssinus* in NC/Nga mice

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Abstract

Mite antigen has been considered to play important roles in the development of atopic eczema/dermatitis syndrome (AEDS). In the present study, we attempted to induce an AEDS-like skin lesion in mice using *Dermatophagoides pteronyssinus* crude extract (DPE) as an antigen and performed pathophysiological evaluations. Ears of mice were tape-stripped and DPE was painted 3 times a week. Eczematous skin lesion and ear swelling were apparent in NC/Nga mice treated with DPE after 2 weeks, whereas neither skin lesion nor ear swelling were observed in BALB/c mice even after 30 days. Histological evaluation demonstrated that edema, epidermal hyperplasia and the accumulation of inflammatory cells were apparent in the ears of DPE-treated NC/Nga mice. In contrast to skin lesion and ear swelling, total serum IgE levels were increased in both NC/Nga and BALB/c mice. Treatment with DPE also increased auricular lymph node weight in both NC/Nga mice and BALB/c mice. To further characterize, we analyzed cytokine mRNA expression in ears and lymph nodes of DPE-treated NC/Nga mice. Increased expression of IL-4 and TNF-α mRNA was observed in both ears and lymph nodes of NC/Nga mice treated with DPE. Additionally, there was no change in the responsiveness of BALB/c mice to DPE treatment by adaptive transfer of serum from DPE-treated NC/Nga mice to BALB/c mice. Taken together, our results indicate that eczematous skin lesion and ear swelling caused by repeated application of DPE in NC/Nga mice has a Th2-dominant background and that inflammation is involved in this process. The animal model of AEDS established in this report may be used to investigate the pathogenesis of AEDS and evaluate the potential therapeutic agents for AEDS.

Keywords: *Dermatophagoides pteronyssinus*; Atopic eczema/dermatitis syndrome; IgE; IL-4; TNF-α

1. Introduction

Atopic eczema/dermatitis syndrome (AEDS) is one of the most common and severe skin diseases, which is characterized by eczematous skin lesion and IgE hyperproduction [1]. Both genetic and environmental factors are involved in the occurrence of this disease [2]. The prevalence of AEDS is increasing especially in industrialized countries because of frequent exposure to harmful environmental pollutants from industry [3,4]. Although AEDS is a severe health problem in developed countries, its pathogenesis is not clearly understood.

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Therefore, appropriate animal models have been desired to investigate the pathogenesis of AEDS and explore new therapeutic agents for the treatment of this disease. There are several mouse models of AEDS available now. NC/Nga mouse model is a representative of the animal model of AEDS. NC/Nga mice develop AEDS-like skin lesions spontaneously in a conventional condition and shows elevated levels of serum IgE [5]. The IL-18-transgenic mouse model has been known as one of the closest transgenic mouse model of human AEDS [6]. Although each of these models has usefulness, it is reported that all of these models have significant disadvantages [7]. Therefore, the development of new animal models for atopic dermatitis has been required.

The most important allergens associated with human AEDS are house dust mite allergens [8]. *Dermatophagoides* species, including *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, are the most common house dust mites present in the environment. The geological distribution of *Dermatophagoides* species are known to be related to the climate. *D. pteronyssinus* prefers humid climates and found abundantly in islands and coastal areas [9,10]. In contrast, *D. farinae* is more common than *D. pteronyssinus* in drier inland areas [10,11]. Although a couple of groups established mouse models of AEDS by topical application of *Dermatophagoides* extract, they all used the extract of *D. farinae* instead of *D. pteronyssinus* [1,12]. The aim of this study, therefore, was to establish a mouse model of AEDS by repeated topical application of *D. pteronyssinus* crude extract in mice and compare with previously reported AEDS model established by topical application of the extract of *D. farinae*. Here, we provide a relatively easy, fast and consistent way to develop AEDS-like skin lesion in NC/Nga mice.

2. Materials and methods

2.1. Reagents and animals

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. *D. pteronyssinus* crude extract (DPE, Woongbee Meditech, Inc., Seoul, Korea) was used as an antigen. DPE was dissolved in phosphate buffered saline (PBS) containing 0.5% Tween 20 as descried previously [1]. Five-week-old female NC/Nga and BALB/c mice were purchased from Charles River Japan (Kanagawa, Japan) and Orient Bio, Inc. (Seoul, Korea), respectively, and acclimated for 1 week before use.

2.2. Induction of AEDS-like skin lesions by repeated topical application of DPE

Induction of AEDS-like skin lesions using mite antigen was performed according to the method of Gao and coworkers [1] with several major modifications. The dorsal surfaces of both ears were tape-stripped three times and 25 μl of 10 mg/ml DPE solution was painted onto the surface of each ear. Tape-stripping and DPE painting were conducted three times a week for 3 weeks, and ear thickness was measured using a dial thickness gauge (Mitutoyo Corporation, Kanagawa, Japan) immediately before each tape-stripping. The vehicle solution was painted instead of DPE solution in control mice. At day 21, blood samples were collected by orbital puncture and used for the measurement of serum immunoglobulin levels. After blood collection, ears were removed and used for the evaluation of cytokine mRNA expression and histological observations. Auricular lymph nodes were also removed, weighed and used for the analysis of mRNA expression of cytokines. In adaptive transfer experiment, isolated sera were intravenously injected to the tail vein of BALB/c mice.

2.3. Detection of serum immunoglobulin levels

Immunoglobulin levels were measured using enzyme-linked immunosorbent assay kit (Bethyl Laboratories, Inc., Montgomery, TX, USA) according to the manufacturer's instructions. Briefly, capture antibodies were plated in Nunc C bottom immunoplate contained in the kit and incubated 1 h at room temperature. After washing the wells with washing solution (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0) three times, 200 μl of blocking solution (50 mM Tris, 0.14 M NaCl, 1% BSA, pH 8.0) was plated in each well. After 30 min incubation at room temperature, the wells were washed three times with washing solution, serum samples and standards were diluted (serum samples were diluted 20-fold for IgE detection and 5000-fold for IgG, IgG_1 and IgG_{2a} detection), plated in the wells and incubated for 1 h. After washing the wells with washing solution, horseradish peroxidase-conjugated detection antibodies were diluted 5000-fold with conjugate diluent (50 mM Tris, 0.14 M NaCl, 1% BSA, 0.05% Tween 20, pH 8.0), transferred to each well and incubated for 1 h. After washing three times with washing solution, the enzyme reaction was initiated by adding substrate solution and the plate was kept for 30 min at room temperature in a dark place. The reaction was terminated by adding 2 M H_2SO_4 and the absorbance was measured.

2.4. Evaluation of cytokine mRNA expression

The ears were frozen in liquid nitrogen, grinded and suspended in TRIzol® Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). The lymph nodes were homogenized in TRIzol® Reagent using tissue tearer (Biospec Products, Inc., Bartlesville, OK, USA). Total RNA was isolated as described previously [13]. The expressions of the mRNA transcripts of IL-4 (forward primer: 5′-GAATGTACCAGGAGCCATATC-3′, reverse primer: 5′-CTCAGTACTACGAGTAATCCA-3′), TNF-α (forward primer: 5′-CCTGTAGCCCACGTCGTAGC-3′, reverse primer: 5′-TTGACCTCAGCGCTGAGTTG-3′) and β-actin (forward primer: 5′-TGGAATCCTGTGGCATCCATGAAAC-3′, reverse primer: 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′) were