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Analysis of T cell receptor (TCR) BV-gene clonotypes in NC/Nga mice developing dermatitis resembling human atopic dermatitis

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T cell receptor;
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Summary

Background: Our previous study showed that T cells in skin lesions of human atopic dermatitis (AD) had oligoclonal accumulation, indicating the involvement of antigen-specific immune reactions at those sites. Recently, NC/Nga mice, which develop skin lesions similar to AD, have been proposed as a model for that disease.
Objective: To clarify whether NC/Nga mice are suitable as a model for human AD from the viewpoint of their antigen-specific immune responses.
Methods: Reverse transcription-polymerase chain reaction (RT-PCR) and single strand conformation polymorphism (SSCP) analyses were conducted to detect TCR BV genes of clonally expanded T cells derived from NC/Nga mice at an early phase of the AD-like dermatitis, at a late phase of the dermatitis, and with no AD-like dermatitis.
Results: (1) T cells with TCR BV 7, 10 and 17 reside in the skin of NC/Nga mice without the AD-like dermatitis. (2) T cells with these BV genes contain oligoclonal accumulations, however, expanded T cell clonotypes are also detected in the spleen and exist constantly during the course of the AD-like dermatitis. (3) Development of the AD-like dermatitis is associated with additional oligoclonal expansion/accumulation of T cells with TCR BV 2, 4 and 6 genes. (4) Progression of the AD-like dermatitis is associated with further oligoclonal expansion/accumulation of T cells with the TCR BV 14 gene. (5) Some of the expanded TCR clonotypes are common between the individual mice and between early and late phases.

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Conclusions: Taking these data together with the previous human AD studies, NC/Nga mice seem to be an appropriate model for human AD.

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease with infiltrations of lymphocytes in the skin lesions. T cells as well as monocytes/macrophages account for a considerable part of those infiltrating lymphocytes [1—3]. In our previous study, T cells in the skin lesions of human AD showed oligoclonal accumulation identified by single strand conformation polymorphism (SSCP) analysis of T cell receptor (TCR) B chain-gene products, indicating the involvement of antigen-specific immune reactions at the sites of AD lesions [4,5].

Recently, NC/Nga mice, which develop spontaneous dermatitis, which resembles human AD, have been used as an animal model for that disease in evaluating possible therapeutic drugs. NC/Nga mice show hypersensitivity to irradiation, anaphylaxis to ovalbumin, anemia and often develop chronic glomerulonephritis as they age. Interestingly, this strain of mice develops dermatitis mainly on their faces 7—8 weeks after birth only under conventional environmental conditions and not under specific pathogen-free (SPF) conditions. This dermatitis is associated with thickening of the epidermis, dander, incrustation and bleeding, and becomes progressively severe until 13—14 weeks of age. After that, the progression of the dermatitis become somehow slow but still continues until about 17 weeks of age. NC/Nga mice show elevated levels of serum IgE immunologically, and increased numbers of mast cells and eosinophils in the dermis and impaired skin barrier function histologically. The serum IgE levels start to increase about 6 weeks of age and progressively increase until about 12 weeks of age [6—9]. Since these features closely resemble those of human AD, NC/Nga mice have been proposed as a model for human AD.

T cells in skin lesions of human AD show oligoclonal accumulation, indicating the involvement of antigen-specific immune reactions at sites of dermatitis [4,5]. If T cells in the skin lesions of NC/Nga mice display a similar behavior to those of human AD, NC/Nga mice would be a good model for human AD from the viewpoint of cellular immunological mechanisms. However, whether accumulation of T cells in the skin lesions of NC/Nga mice results from antigen-specific immune reactions has not been investigated so far. We thus, attempted in this study, to investigate the T cell clonality in skin lesions of NC/Nga mice.

Specifically, we used SSCP analysis of TCR B-chain-gene products as we established previously [10—12]. This enables us to distinguish nucleotide sequence differences in the diversity regions of TCR B chains. With this technique, we confirmed the adequacy of NC/Nga mice as a model for human AD from the viewpoint of antigen-specific immune responses at the molecular level.

2. Materials and methods

2.1. Mice

Male NC/Nga mice were purchased from Japan SLC Inc. (Shizuoka, Japan). The mice were housed and fed under conventional conditions or under SPF conditions until used.

2.2. Histological examination

Hematoxylin-eosin (H-E) staining was conducted on dorsal skin lesions from NC/Nga mice bred in conventional conditions and in normal skin from NC/Nga mice bred in SPF conditions.

2.3. cDNA preparation and polymerase chain reaction (PCR)

Skin and spleen samples were individually homogenized and immediately processed for RNA preparation. Total RNA was isolated from the samples by the acid guanidium thiocyanate-phenol-chloroform method [10]. The extracted RNA was then converted to cDNA, using reverse transcriptase and random hexamer oligonucleotide priming at 42 °C for 1 h using a first strand cDNA synthesis Kit (Roche Diagnostics Co., Indianapolis, IN). PCR was performed using 20 ng of the cDNA and 50 pmol each of variable region (VB)-specific primers and a common CB primer for 40 cycles (94 °C for 1 min, 60 °C 1.5 min, and 72 °C for 1.5 min) in a thermocycler using Pure TaqTM Ready-To-GO PCR Beads (Amersham Biosciences Corp., Piscataway, NJ). The nucleotide sequences of the primers used are as follows:

BV 1, 5′-TTCGAAATGAGACGGTGCCC [13];