

Institute of DNA Medicine

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General Summary

Our research interests have focused on the analysis of the basic immune system, which protects us from a number of diseases, and of immune disorders, such as hypersensitivity diseases and autoimmune diseases.

Research Activities

A single dose of interleukin 31 causes continuous itch-associated scratching behaviour in mice

Interleukin (IL) 31 is a T-cell-derived cytokine that induces severe pruritus, hair loss, and dermatitis and is involved in allergic diseases, such as atopic dermatitis and bronchitis. We investigated the effects of a single dose of mouse IL-31 on scratching behavior in comparison with scratching behavior induced by spontaneous skin lesions or serotonin (5-HT) in NC/Nga and BALB/c mice. About 3 hours after administration, intradermal injection of IL-31 caused a gradual increase in long-lasting scratching (LLS, more than 1.5 seconds), which was followed by a gradual decrease over 24 hours after administration. Intradermal injection of IL-31 significantly increased the total LLS counts/24 hours but did not increase short-lasting scratching (SLS, 0.3-1.5 seconds). In NC/Nga mice with skin lesions, the LLS counts, but not the SLS counts, were significantly higher than those in NC/Nga mice without skin lesions. We also investigated 5-HT-induced scratching in BALB/c mice; we found that SLS counts, but not LLS counts, increased immediately after the injection and then decreased to baseline after 20 minutes. These results suggest that IL-31 participates in the sensation of itching and promotes scratching behavior in NC/Nga mice with skin lesions, an animal model of atopic dermatitis.

Cross-reactivity at the T-cell and B-cell levels within the Cupressaceae family and Taxodiaceae subfamily in patients with Japanese cedar pollinosis

Allergens of the Cupressaceae family and Taxodiaceae subfamily are a major cause of pollinosis in several geographic areas. A comparison of immunoglobulin E (IgE) epitope regions has shown that allergens from several taxa cross-react. However, the cross-reactivity at the T-cell level within the Cupressaceae family and the Taxodiaceae subfamily has not been analyzed in detail.

We evaluated the cross-reactivity at the T-cell and B-cell levels between Cupressaceae and Taxodiaceae allergens in patients with Japanese cedar pollinosis. Crude extracts of Cupressaceae and Taxodiaceae pollens were used as allergens for analysis. Proliferative responses to the allergens of peripheral blood mononuclear cells from patients were

examined for cross-reactivity at the T-cell level, and measurement of CD203c expression on basophils was used to examine allergenic cross-reactivity at the B-cell level. Cry j 1-specific T-cell lines were also used to analyze cross-reactivity at the T-cell level among these families.

Patients with Japanese cedar pollinosis were divided into 2 groups: those who showed reactions to all allergens tested within the Cupressaceae family and Taxodiaceae subfamily and those who showed reactions to some allergens at the T-cell level. The existence of a common T-cell epitope among the Cupressaceae family and the Taxodiaceae subfamily, which was a major human T-cell epitope in Cry j 1 in patients with Japanese cedar pollinosis, was identified with a Cry j 1 p211-225-specific T-cell line. Even if the cross-reactivity to each allergen was not found at the T-cell level, serum IgE antibodies showed cross-reactivity to all allergens tested within the Cupressaceae family and Taxodiaceae subfamily in patients with Japanese cedar pollinosis.

A common T-cell epitope in group 1 allergens among the Cupressaceae family and Taxodiaceae subfamily was identified for the first time. The cross-reactivity at the T-cell level between Cupressaceae and Taxodiaceae species was determined depending on whether T-cells of a patient could recognize peptides of the common T-cell epitope, which was restricted by the HLA haplotype of the patient. On the other hand, serum IgE antibodies of the patients showed cross-reactivity to all allergens of Cupressaceae family and the Taxodiaceae subfamily, because the group 1 or 2 allergens are structurally similar among the Cupressaceae family and the Taxodiaceae subfamily.

These results suggest that symptoms in patients with Japanese cedar pollinosis develop at the T- or B-cell level in response to allergens of the Cupressaceae family and the Taxodiaceae subfamily.

Enhancement of cytotoxic T-lymphocyte induction with cationic liposome by the modification of N-glycan structure

We have developed an adjuvant inducing cytotoxic T lymphocytes (CTLs) via cross-presentation with a cationic liposome. After ovalbumin (OVA) protein and the adjuvant were mixed and administered to mice, the CTLs against the OVA epitope were induced within 5 days independently of helper T cells. Also, CTL-inducing activity was not decreased in TLR-2/4/9 knockout mice.

With this adjuvant, we compared the CTL-inducing activity of OVA derivatives with different N-glycan structures. Mice were immunized with this adjuvant mixed with chicken OVA, OVA expressed in *Escherichia coli*, or OVA expressed in HEK-293F cells, and then the activity of CTL induction was analyzed.

All antigens had the ability to induce CTLs, but the activities were different. It is well known that, the structures of N-glycan differed among secreted and unsecreted proteins when secretory proteins were expressed in HEK-293 cells. Hence, the unsecreted proteins were highly mannosylated, and we reproduced the N-glycan structure of OVA with an inhibitor of N-glycan modification. The secretion of modified OVA was not inhibited, but the ability to induce CTLs with a cationic liposome was enhanced through the modification of N-glycan.

Because the modified OVA without adjuvant failed to induce CTLs, comparing the effects

of N-glycan modification on the basis of cell-membrane receptors is difficult. However, after uptake of OVA by antigen-presenting cells, there may be some mechanism to induce cross-presentation with cationic adjuvants inside cells.

Publications

Arai I, Tsuji M, Takeda H, Akiyama N, Saito S.

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Saito S, Yui N, Aoki K. Amount of ultraviolet

rays in summer mountains (in Japanese). *Nihon Sangaku Bunka Gakkai Ronshu.* 2013; **11**: 47-52.