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DNA vaccination against Japanese cedar pollinosis in dogs suppresses type I hypersensitivity by controlling lesional mast cells

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Abstract

Sensitization to allergens of Japanese cedar pollen is known to cause canine atopic dermatitis as approximately 10% of atopic dogs in Japan were positive to the pollen allergen. Among the two major allergens of Japanese cedar pollen, since Cry j 1 is more important than Cry j 2 as an antigen to increase IgE in atopic dogs sensitized to Japanese cedar pollen, Cry j 1 can be a target for immunotherapy. In our study, efficacy of DNA vaccination with a plasmid containing the gene of a major allergen of Japanese cedar (*Cryptomeria japonica*, CJ) pollen, Cry j 1, was examined using a dog model experimentally sensitized to CJ pollen allergen. Cry j 1 DNA plasmid and a vector plasmid (pCAGGS) were injected into six dogs and three dogs, respectively, five times with an interval of 1.5 month. After the treatment with Cry j 1 DNA plasmid, production of IgE against Cry j 1 decreased in four of the six dogs in the treatment group, whereas it increased in the three dogs of the control group. The reactivity to the pollen allergen in intradermal testing and provocation testing were obviously reduced in the treatment group, but not in the control group. The number of mast cells in alveolar area of the lung in the treatment group was smaller than that in the control group. Cry j 1 DNA plasmid was also injected into three atopic dogs sensitive to Cry j 1, resulting in improvement of clinical signs in the pollination season. These findings indicated that Cry j 1 DNA plasmid could regulate mast cell-mediated reaction against Cry j 1, which could be an alternative and effective treatment for CJ pollinosis.

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1. Introduction

It is known that allergy is induced by over-reaction of immune system against exogenous antigens, resulting in hypersensitivity. Although there are four

types of hypersensitivity against allergens, the most important is type I hypersensitivity which is the immediate reaction mediated by IgE and mast cells, consequently inducing inflammation by various cytokines up-regulated by the IgE-mediated reaction and causing clinical signs such as rhinitis, conjunctivitis, and bronchitis. The type I hypersensitivity can also induce subsequent late antibody- and/or delayed

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lymphocyte-mediated reactions which makes the pathogenesis more complex to understand. Thus, the initial type I allergic reaction is the principal intended target of immunosuppressive therapy to reduce complex hypersensitivity reactions.

2. Japanese cedar pollinosis in dogs in Japan

It has been widely known that people in Japan are affected with Japanese cedar pollinosis which is a type I hypersensitivity against Japanese cedar pollen. Clinical signs of Japanese cedar pollinosis in humans are characterized by conjunctivitis and rhinitis occurring in spring, which is the pollen season for Japanese cedar (Ogino et al., 1990). In atopic dogs, sensitization to Japanese cedar pollen was identified with positive results against the pollen allergen in intradermal testing and IgE testing (Sasaki et al., 1995). In our previous study, approximately 10% of atopic dogs in Japan were positive to the pollen allergen, although house dust mites were the most common allergens (Masuda et al., 2000a). These dogs showed clinical signs of atopic dermatitis during spring and had typical reactions of type I hypersensitivity, including increased concentration of antigen-specific IgE in serum, positive results in intradermal testing, and histamine release from blood basophils against Japanese cedar pollen antigen (Masuda et al., 2000b), similar to those in human patients with Japanese cedar pollinosis.

There are two major allergens of Japanese cedar pollen, called Cry j 1 (Yasueda et al., 1983) and Cry j 2 (Sakaguchi et al., 1990), against which IgE was identified in human patients with Japanese cedar pollinosis. We have previously measured serum concentration of anti-Cry j 1 IgE and anti-Cry j 2 IgE in the dogs sensitized to Japanese cedar pollen and found that all the dogs had increased concentration of anti-Cry j 1 IgE in serum (Masuda et al., 2000c). The proportion of dogs examined that had increased anti-Cry j 2 IgE in serum was 37% (Masuda et al., 2000c). These findings suggest that Cry j 1 is more important than Cry j 2 as an antigen to increase IgE in atopic dogs sensitized to Japanese cedar pollen. The dogs sensitized to Cry j 1 increased the serum concentration of IgE against Cry j 1 in spring the season of pollination and decreased the level in fall, out of the

pollination season as indicated by development of clinical signs of atopic dermatitis (Masuda et al., 2002). Lymphocyte blastogenic responses to Cry j 1 in the dogs were similar to those against crude antigen of Japanese cedar pollen, supporting that Cry j 1 might play an important role in development of allergic reaction and clinical signs of atopic dermatitis in the dogs sensitized to Japanese cedar pollen (Masuda et al., 2002). Therefore, it should be useful to develop immunotherapy against Cry j 1 hypersensitivity for these dogs sensitized to Cry j 1.

3. DNA vaccination with a plasmid immunization

Vaccination with DNA has become an alternative strategy to induce hyposensitization for allergic diseases, since it was found to increase IgG against a protein that was produced in vivo in response to allergen DNA sequence inserted into a plasmid (Raz et al., 1996; Spiegelberg et al., 1997). Continuous production of allergen protein in vivo is an advantage of using DNA vaccination in immunotherapy, leading to reduced frequency of injections and increased efficacy of hyposensitization and also effective induction of Th1 response can be expected when the CpG motif is contained in plasmids (Bohle, 2002; Heeg and Zimmermann, 2000; Krieg, 2002).

We have developed a plasmid DNA vaccine encoding the Cry j 1 DNA sequence, which was confirmed to induce IgG antibody against Cry j 1 in mice (Toda et al., 2000). In order to further confirm suppressive effects of the plasmid DNA vaccination on allergic reaction, dogs experimentally sensitized to Japanese pollen were used. The sensitized dogs showed positive reactions to antigen of Japanese cedar pollen in intradermal testing, IgE testing (Yamashita et al., 2000), and lymphocyte blastogenic responses with skewed responses to Th2-type cytokine production (unpublished data). The dogs also showed clinical signs of allergic bronchitis when exposed to the pollen allergen through the trachea, resulting in increased respiratory rates.

The plasmid containing Cry j 1 gene was injected into three dogs five times at 500 µg with an interval of 1.5 months for 7.5 months. At the end of the study, intradermal testing showed that sensitivity to the

pollen allergen was decreased in the group treated with the plasmid DNA vaccination, although serum concentration of IgE against Japanese cedar pollen and Cry j 1 was not dramatically decreased. In the bronchial exposure to the pollen allergen, bronchial hyper-reactivity was suppressed in the treatment group. It was also found that the number of mast cells decreased in the lung in the treatment group. These results might indicate that the plasmid DNA vaccination controlled mast cell biology rather than modifying lymphocyte activity such as production of IgE. The effects finally resulted in decrease of the sensitivity to the pollen allergen in type I hypersensitivity. The effective mechanism of the plasmid DNA vaccination should be further investigated from the aspect of regional rather than systemic immune response.

The plasmid was also injected into three atopic dogs sensitized to Cry j 1 in order to examine clinical effects. A total of five injections at 500 µg of the plasmid were performed in the femoral muscle of dogs during the period from January to May. In these dogs, atopic dermatitis was suppressed successfully during the pollen season; however, serum concentration of IgE against Japanese cedar pollen were not decreased. Lymphocyte blastogenic responses to the pollen antigen were decreased to the background levels in the atopic dogs. Although 4 years have passed since the plasmid vaccination, there has not been recurrence of clinical signs in atopic dogs.

4. Conclusion

Allergy against Japanese cedar pollen was found in atopic dogs in Japan. Cry j 1 plays an important role in development of atopic dermatitis by inducing type I hypersensitivity as in human patients with Japanese cedar pollinosis. The canine hypersensitivity can be controlled by immunotherapy using plasmid DNA vaccination. Thus, it can be concluded that dogs may be useful as an animal model of human allergic diseases.

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