

## Institute of DNA Medicine

### Department of Molecular Immunology

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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

##### *Pleiotropic function of interleukin 31*

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. To investigate the function of IL-31, IL-31 transgenic mice were created in our laboratory. In addition to scratching behavior and hair loss as reported previously, enhancement of the serum immunoglobulin (Ig) E level was observed in the IL-31 transgenic mice. Moreover, these pleiotropic functions were verified by the administration of IL-31 into normal mice.

Furthermore, to investigate the function and the locations of IL-31 or the IL-31 receptor, 2 strains of IL-31 or IL-31 receptor (IL-31R) knockout/*LacZ* knockin mice were generated. In *IL-31R<sup>+/lacZ</sup>* knockin mice 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside staining was limited to the hair matrix. To produce offspring with a genetic identity for the analysis of the pleiotropic functions of IL-31, the heterozygous mouse will be backcrossed 6 or more times into the C57B/6J genetic background.

We tested the ability of *IL-31R<sup>lacZ/lacZ</sup>* knockin mice to respond to IL-31 delivered via intradermal injection (10  $\mu$ g per day for 14 days) compared with the responses of heterozygous littermate control mice. Thus, neither alopecia nor pruritus developed in *IL-31R<sup>lacZ/lacZ</sup>* knockin mice in response to IL-31 over the 14-day course of treatment.

##### *A rice-based edible vaccine expressing Japanese cedar pollen allergens induces oral tolerance in Japanese monkeys with Japanese cedar pollinosis*

Japanese cedar (*Cryptomeria japonica* [CJ]) pollinosis affects more than 30% of the Japanese population and is, thus, one of the most common diseases in Japan. Furthermore, CJ pollinosis has been found to occur naturally in Japanese macaques (*Macaca fuscata*), which show symptoms similar to those of human patients. Plants have recently been recognized as a form of bioreactor for the cost-effective production of large-scale recombinant proteins. The edible tissue of plants provides the further significant benefit of being a simple method of mucosal delivery of vaccines without the need for complicated purification steps.

Our previous study showed that oral administration to mice of transgenic rice seeds that have accumulated high concentrations of polypeptides derived from CJ pollen allergens reduces the mice's serum IgE levels and T-cell proliferative responses to CJ allergens, proving the efficacy of oral immunotherapy for the treatment of pollinosis.

In this study, the transgenic rice plants that had accumulated high concentrations of JC allergens were used for oral immunotherapy for CJ pollinosis in macaques. Five macaques with CJ pollinosis were fed once a day with 20 g of the rice seeds containing about 50 to 60 mg of allergens for 3 months. No side effects, such as urticaria, dyspnea, vomiting, and weight loss, were observed during immunotherapy. One and a half months after the start of feeding, proliferative responses of T cells to JC allergens in 4 of 5 macaques were significantly inhibited compared with those in macaques at the start of feeding. However, their T-cell responses to CJ allergens were restored 1 month after the end of feeding. On the other hand, in healthy macaques without CJ pollinosis, the side effects and the induction of immune responses to CJ allergens were not observed after oral administration of transgenic rice seeds.

These results indicate that oral immunotherapy with transgenic rice seeds is a safe and effective treatment for pollinosis.

#### *Induction of cytotoxic T lymphocytes with liposome-based adjuvants*

Vaccines that induce specific cytotoxic T lymphocytes (CTLs) against tumors and pathogens appear to be a promising approach to treating these diseases. To make efficient T-cell vaccines, several types of liposome were developed to induce CTLs specific to antigens. For the clinical use of these vaccines, the absorption of antigen into liposome and the stabilization of these complexes are always troublesome.

To overcome these problems, we have developed liposome-based synthetic adjuvants, which enable absorption when mixed with antigens just before injection, and analyzed the properties of the adjuvants with chicken ovalbumin (OVA) as a model antigen. The OVA-specific CTLs were efficiently induced within 4 days after a single immunization and were maintained for more than 2 weeks. The induced CTLs were antigen dose-dependent and showed more than 95% killing activity with an *in vitro* CTL assay. These CTL activities were also confirmed with an *in vitro* chromium-release assay and with tumor growth inhibition *in vivo*.

#### **Publications**

**Kamada M, Ikeda K, Fujioka K, Akiyama N, Akiyoshi K, Inoue Y, Hanada S, Yamamoto K, Tojo K, Manome Y.** Expression of mRNAs of urocortin and corticotropin-releasing factor receptors in malignant glioma cell lines. *Anticancer Res.* 2012; **32**: 5299-307.

#### **Reviews and books**

**Saito S.** Oral immunotherapy with transgenic rice seed containing Japanese cedar pollen allergens and future prospect (in Japanese). *Allergy no Rinsho.* 2013; **33**: 47-51.