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Original article

Efficacy of oral immunotherapy with a rice-based edible vaccine containing hypoallergenic Japanese cedar pollen allergens for treatment of established allergic conjunctivitis in mice

Ken Fukuda [a, *], Waka Ishida [a], Yosuke Harada [a, c], Yuhya Wakasa [b], Hidenori Takagi [b], Fumio Takaiwa [b], Atsuki Fukushima [a]

[a] Department of Ophthalmology and Visual Science, Kochi Medical School, Kochi, Japan
[b] Plant Molecular Farming Unit, Division of Biotechnology, Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, Ibaraki, Japan

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ABSTRACT

Background: We have previously shown that prophylactic oral administration of transgenic rice seeds expressing hypoallergenic modified antigens suppressed the development of allergic conjunctivitis induced by Japanese cedar pollen. We have now investigated the efficacy of oral immunotherapy with such transgenic rice for established allergic conjunctivitis in mice.

Methods: BALB/c mice were sensitized with two intraperitoneal injections of Japanese cedar pollen in alum, challenged with pollen in eyedrops, and then fed for 16 days with transgenic rice seeds expressing modified Japanese cedar pollen allergens Cry j 1 and Cry j 2 or with nontransgenic rice seeds as a control. They were then challenged twice with pollen in eyedrops, with clinical signs being evaluated at 15 min after the first challenge and the eyes, blood, spleen, and lymph nodes being isolated at 24 h after the second challenge.

Results: The number of eosinophils in the conjunctiva and the clinical score for conjunctivitis were both significantly lower in mice fed the transgenic rice than in those fed nontransgenic rice. Oral vaccination with transgenic rice seeds also resulted in a significant increase in the production of IFN-γ by splenocytes, whereas it had no effect on the number of $CD4^+CD25^+Foxp3^+$ regulatory T cells in the spleen or submandibular or mesenteric lymph nodes.

Conclusions: Oral administration of transgenic rice seeds expressing hypoallergenic allergens ameliorated allergic conjunctivitis in the established setting. Such a rice-based edible vaccine is potentially both safe and effective for oral immunotherapy in individuals with allergic conjunctivitis.

Introduction

Pollinosis including allergic conjunctivitis is one of the most common diseases worldwide. Japanese cedar (*Cryptomeria japonica*) pollinosis is a predominant allergic condition in Japan, with a prevalence of >25%.[1] Most treatments for allergic conjunctivitis are not antigen specific but rather involve the administration of antiallergy eyedrops that contain a mast cell stabilizer or antihistamine (or both agents) to relieve symptoms. Allergen-specific immunotherapy is the only available curative treatment for allergic diseases in which the clinical effects persist over the long term. Such immunotherapy was initially performed by SCIT that required frequent hospital visits for injections and was thus highly inconvenient for the patient. The subsequent introduction of SLIT, which is administered at home by patients themselves without pain, solved the inconvenience problem. However, the rate of discontinuation of SLIT is high, with <15% of patients who initiate the treatment persisting with it for >2 years.[2] Given that such early discontinuation of treatment reduces the clinical efficacy of immunotherapy, this poor compliance is an important clinical problem.

Oral administration of staple foods engineered to express allergens is one possible means of antigen delivery for immunotherapy of allergic diseases that would address both issues of

* Corresponding author. Department of Ophthalmology and Visual Science, Kochi Medical School, Kohasu, Oko-cho, Nankoku, Kochi 783-8505, Japan.
E-mail address: k.fukuda@kochi-u.ac.jp (K. Fukuda).
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[c] Present address: Department of Ophthalmology and Visual Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan.

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convenience and compliance. To develop more effective, safer, and convenient immunotherapy for allergic diseases, we have prepared rice-based edible vaccines that express various antigens including those derived from Japanese cedar pollen, birch pollen, or house dust mites.[3] We recently established transgenic rice expressing the entire molecules of the major Japanese cedar pollen allergens Cry j 1 and Cry j 2 after their molecular fragmentation or shuffling in order to treat a broader range of patients with different genetic backgrounds.[4] Furthermore, we also recently showed that the development of Japanese cedar pollen–induced allergic conjunctivitis is suppressed by prophylactic feeding of such transgenic rice seeds in a mouse model.[5] In the clinical setting, a therapeutic effect of vaccination for established pollinosis is more important than a preventive effect for nonsensitized healthy individuals. We have therefore now examined whether oral immunotherapy with transgenic rice seeds expressing these hypoallergenic modified antigens suppresses established cedar pollen–induced allergic conjunctivitis in mice.

Methods

Mice

Inbred wild-type BALB/c mice were obtained from Japan SLC (Hamamatsu, Shizuoka, Japan) and were maintained under specific pathogen–free conditions at the animal facility of Kochi Medical School. Age- and sex-matched mice were subjected to experiments at 6–12 weeks of age. This study was approved by the Committee for Care and Use of Laboratory Animals at Kochi University (permit no. I-77) and was performed in strict accordance with the Statement on the Use of Animals in Ophthalmic and Vision Research of the Association for Research in Vision and Ophthalmology.

Transgenic rice

The major Japanese cedar pollen allergens Cry j 1 and Cry j 2 were deconstructed by fragmentation and molecular shuffling, respectively. Transgenic rice that accumulates such modified forms of Cry j 1 and Cry j 2 in the edible portion (endosperm) of the seed was generated by transformation as described previously.[4] Cry j 1 was thus divided into three overlapping fragments that were expressed as fusion proteins with rice seed storage glutelins (GluA2, GluB1, and GluC), whereas Cry j 2 deconstructed by shuffling was expressed as a secretory protein by attachment of an NH_2-terminal signal peptide and COOH-terminal endoplasmic reticulum retention signal (Lys-Asp-Glu-Leu). The modified antigens are deposited in endoplasmic reticulum–derived protein bodies and are thereby rendered resistant both to hydrolysis by intestinal enzymes and to harsh environments. The transgenic rice is thus suitable for oral delivery of the antigens to the mucosal immune system in gut-associated lymphoid tissue.[6]

Feeding and sensitization of mice

Mice had access to a powder diet and drinking water ad libitum. Experimental allergic conjunctivitis was induced by a modified version of a previously described protocol (Fig. 1A).[5,7] In brief, mice were injected intraperitoneally twice with 0.2 mg of Japanese cedar pollen (Hayashibara, Okayama, Japan) mixed with alum (2.5 mg), with an interval of 7 days between injections. Six days after the second sensitization, both eyes of each mouse were challenged with Japanese cedar pollen in PBS (1.2 mg per 2 μl per eye) and clinical signs such as lid swelling, tear production or discharge, chemosis, and redness were evaluated 15 min later in a double-blind manner on the basis of previously described criteria.[8] The

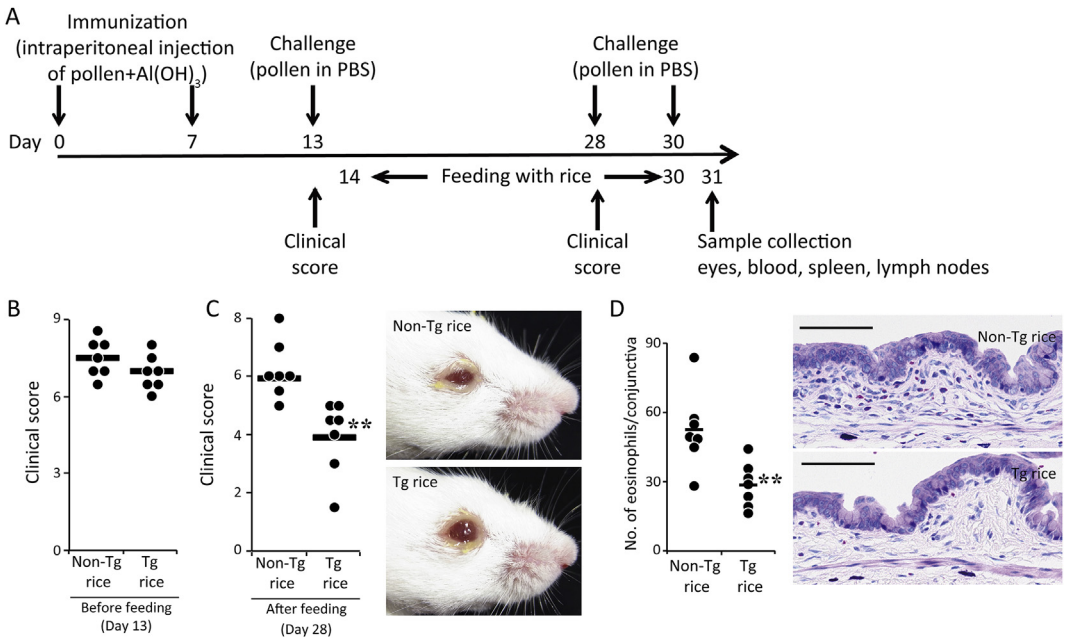

Fig. 1. Therapeutic feeding with transgenic rice suppresses both early-phase signs of and late-phase inflammation in experimental allergic conjunctivitis. As shown in **(A)**, mice were injected intraperitoneally twice (day 0 and 7) with Japanese cedar pollen grains mixed with alum. On day 13, both eyes of each mouse were challenged with Japanese cedar pollen grains suspended in PBS and clinical scores were evaluated 15 min later **(B)**, with circles representing the mean values for the two eyes of each mouse and the horizontal lines indicating overall median values. The mice were then randomly divided into two groups that were provided each day for 16 days with nontransgenic rice seeds or transgenic rice seeds. All mice were challenged twice with pollen in eyedrops on days 28 and 30. The clinical score was evaluated 15 min after allergen challenge on day 28, with representative photographs also being shown **(C)**. The eyes, blood, spleen, and lymph nodes of the mice were isolated on day 31. Eyes were subjected to histological analysis with Giemsa staining to determine the number of eosinophils in the conjunctiva. Representative photographs for mice fed nontransgenic or transgenic rice are shown in **(D)**; scale bars, 200 μm. Individual circles and bars in **(D)** represent mean values for the number of eosinophils in conjunctival sections from the two eyes of each mouse and the overall median values, respectively. Data are representative of two independent experiments with similar results. $^{**}P < 0.01$ (Student's *t* test) versus the value for nontransgenic rice.

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