Efficacy of transgenic rice containing human interleukin-10 in experimental mouse models of colitis and pollen allergy

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Recombinant human interleukin-10 (hIL-10) is highly expressed in transgenic rice endosperm and forms active homodimers in the ER-derived hIL-10 body. In this study, we examined the preclinical efficacy of transgenic rice accumulating hIL-10 (hIL-10 rice) in vivo experimental mouse models of colitis and pollen allergy. In the group of mice orally fed hIL-10 rice, the development of Japanese cedar pollen allergen-specific IgE and splenic T cell responses were significantly inhibited. In addition, oral feeding of hIL-10 rice showed therapeutic as well as prophylactic efficacy against experimental colitis developed in IL-10-deficient mice. These results indicate the clinical potentiality of hIL-10 rice for the control of inflammatory and allergic disorders through efficient delivery of hIL-10 to the gut-associated lymphoid tissue.

Key words: Colitis, interleukin-10, Japanese cedar pollen allergy, mucosal delivery, rice endosperm.

Interleukin-10 (IL-10) is a therapeutic candidate for the suppression of inflammatory and allergic immune responses (Asadullah et al. 2003; Ouyang et al. 2011; Wang et al. 2014). Previously, recombinant human IL-10 (hIL-10) was expressed in the endosperm of transgenic rice seed (Fujiiwara et al. 2010). Compared to the E. coli- or baculovirus-derived commercial IL-10, rice-produced hIL-10 showed higher biological activities in vitro and lower endotoxin levels, indicating the clinical advantages of hIL-10 expressed in rice endosperm (Fujiiwara et al. 2010).

To increase the accumulation level of hIL-10 in the endosperm, hIL-10 was recently expressed in transgenic rice lines, in which endogenous rice seed storage proteins prolamins were down-regulated by the RNA interference-based silencing technique (Yang et al. 2012). In these lines, synthesized hIL-10 formed novel ER-derived granules, named hIL-10 bodies, leading to an increase in the accumulation level of hIL-10 (Yang et al. 2012). In addition, hIL-10 in rice endosperm formed homodimers, the active form of IL-10, and was resistant to in vitro digestion with pepsin (Yang et al. 2012). These studies suggested the clinical potential of rice endosperm for the production and efficient mucosal delivery of hIL-10 (Yang et al. 2012). In the present study, the preclinical efficacy of transgenic rice accumulating hIL-10 bodies (hIL-10 rice) was examined in vivo experimental mouse models of colitis and Japanese cedar pollen allergy.

A homozygous transgenic rice line, in which the pProless/H-IL-10 plasmid had been integrated into the rice genome (Oryza sativa L. cv. Kitaake), was used in this study (Yang et al. 2012). All experiments were conducted in accordance with the Guideline for the Care and Use of Animals published by the Tokyo Metropolitan Institute of Medical Science, and the experimental protocols were approved by the Animal Use and Care Committee of the Tokyo Metropolitan Institute of Medical Science.

We first examined the efficacy of hIL-10 rice in Japanese cedar pollen allergy model (Figure 1A). Six-week old male BALB/c mice were orally given 80 mg fine powder of hIL-10 rice containing approximately 400 µg hIL-10 or non-transgenic control rice once every 2 days from day 0 to day 8. At day 10, mice were intraperitoneally sensitized with 0.1 mg total protein extract of Japanese cedar pollen (Cosmo Bio) adsorbed on 5 mg Alum (aluminum hydroxide) containing recombinant mouse IL-4 at 0.1 µg per mouse to maximize the induction of allergen-specific IgE responses. At day 17, mice were intraperitoneally boosted with 0.1 mg total protein extract of Japanese cedar pollen adsorbed on 5 mg Alum. At day 31, the levels of serum allergen-specific IgE and splenic T cell responses were examined as described previously (Takagi et al. 2010). Compared to the control group of mice fed...
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non-transgenic rice, the levels of allergen-specific IgE were lower in the group of mice fed hIL-10 rice (Figure 1B). In addition, oral feeding of hIL-10 rice suppressed the allergen-specific T cell proliferative responses when compared to those in the control mice (Figure 1C). These results show that hIL-10 accumulated in rice endosperm is effective for the inhibition of the development of pollen allergen-induced allergic immune responses.

Oral administration of cytokines has been recognized to be safe and effective for modulating immune responses in the treatment of inflammatory, autoimmune, and allergic disorders (Rollwagen and Baqar 1996). In an experimental allergic encephalomyelitis model, the effect of orally fed IL-10 was previously examined using myelin basic protein (MBP) as a model antigen. In agreement with our results, oral administration of IL-10 suppressed MBP-induced proliferative responses of lymph node cells (LNC) (Slavin et al. 2001). Furthermore, mice fed MBP plus IL-10 showed significantly lower levels of LNC proliferation than mice fed MBP alone or IL-10 alone, indicating that orally administered IL-10 functions as a mucosal adjuvant by synergistically enhancing the tolerogenicity of co-administered MBP (Slavin et al. 2001). Thus, biologically active hIL-10 rice may...
be applicable for the effective control of a wide range of autoimmune and allergic disorders, in which oral tolerance can be used as a prophylactic or a therapeutic approach.

In conventional animal facilities, interleukin-10-deficient (IL-10−/−) mice spontaneously develop colitis by two to four months of age (Kuhn et al. 1993; Ostanin et al. 2009). To examine the prophylactic effect of hIL-10 rice in colitis, fine powder of hIL-10 rice mixed with non-transgenic rice at a ratio of 1:9 was fed ad libitum to IL-10+/− mice from 1.5 to 4 months of age (Figure 2A). The daily amounts of rice consumed by each mouse were approximately 2.4 g, which contained approximately 1.2 mg hIL-10. Mice were then sacrificed, and the weight of the spleen and the length of the colon from the ileocecal junction to the anal verge were measured. The colon was then opened longitudinally and gently washed with PBS before weight measurement. The weight of the spleen was lower in mice fed hIL-10 rice than in those fed non-transgenic rice (Figure 2B). The weight and length of colon are important quantitative indexes of colon inflammation (Ostanin et al. 2009). Heavier weight and shorter length of colon indicate severer symptoms of colon inflammation. In Figure 2C, the weight-to-length ratio of the colon is described as a quantitative index of colon inflammation. Compared to the control mice fed non-transgenic rice, the level of the weight-to-length ratio was significantly lower in mice fed hIL-10 rice (Figure 2C). The result indicates that oral feeding of hIL-10 rice effectively inhibits the colitis development in IL-10+/− mice.

Next, to study the therapeutic effect of hIL-10 rice in colitis, fine powder of hIL-10 rice mixed with non-transgenic rice at a ratio of 1:9 was fed ad libitum to IL-10+/− mice from 4 to 5 months of age (Figure 3A). In addition to the weight of spleen, the weight-to-length ratio of the colon was lower in mice fed hIL-10 rice (Figure 3B, 3C). Although the number of mice examined is relatively small (n=4), and the efficacy of hIL-10 rice in the therapeutic model was lower than that in the prophylactic model, these results indicate that oral feeding of hIL-10 rice is effective for the prophylactic and therapeutic control, and that higher dose or longer period of hIL-10 rice feeding may be required for achieving therapeutic treatment of colitis in IL-10+/− mice. We are now studying the effect of hIL-10 rice for the prevention and treatment of colitis in wild type mice as well as in IL-10+/− mice.

Based on animal studies using colitis models such as IL-10−/− mice, IL-10 has long been considered as a candidate for the treatment of human inflammatory bowel disease (IBD); however, the efficacy of systemically-administered IL-10 was not demonstrated in human clinical trials for IBD (Asadullah et al. 2003; Ouyang et al. 2011). Alternative administration strategies, in which IL-10 is delivered to the mucosal inflammatory site, may improve the efficacy of IL-10 for the treatment of IBD (Asadullah et al. 2003; Ouyang et al. 2011). Thus, in addition to the advantages of high-yield production of hIL-10 and protection of hIL-10 from gastric digestion, the results of the present study suggest that hIL-10 rice could be a candidate in clinical trials designed to find effective treatments for intestinal inflammatory diseases (Yang et al. 2012).

Our results further stress the advantages of rice endosperm as a production platform and efficient mucosal delivery vehicle for therapeutic peptides and
proteins (Arcalis et al. 2014; Takagi et al. 2010; Takaiwa 2013; Yang et al. 2012). In light of future clinical applications, un-powdered and cooked rice might be a suitable formulation for human consumption. Therefore, we are planning to examine the effect of heating by cooking on hIL-10 rice grain, and to perform quantitative and mechanistic studies of the clinical effects of hIL-10 rice in inflammatory, autoimmune, and allergic disease models.

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