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Note

Effect of Enzymatic Modification of Dietary Wheat Flour for Reducing Its Allergenicity on Oral Sensitization to and Intestinal Absorption of Ovalbumin

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Increase in plasma immunoglobulin G specific to orally administered ovalbumin in Brown Norway rats was retarded by feeding enzyme-treated wheat flour when compared with untreated flour. Because plasma ovalbumin concentrations after feeding ovalbumin tended to be lower in mice fed enzyme-treated flour than in those fed untreated flour, suppression of ovalbumin absorption may be relevant to retarded sensitization observed in rats.

Key words: hypoallergenic flour; ovalbumin; allergen absorption; oral sensitization

Several food-processing techniques have been applied to foods in order to eliminate their allergenic proteins or to reduce their levels. Watanabe *et al*. proposed a method for preparing hypoallergenic wheat flour by enzymatic modification.[1] This procedure involves the digestion of wheat flour with cellulase and actinase, and successfully reduced the allergenicity.[1] In addition, it has been reported that ingesting this hypoallergenic flour prevented gluten-specific allergic airway inflammation in Brown Norway rats by inducing oral tolerance.[2] More recently, Tesaki *et al*. reported that hypoallergenic flour showed inhibitory activity against intestinal permeation of ovalbumin (OVA) in an *in vitro* model by using a Caco-2 cell monolayer.[3] Furthermore, the activity was found in the cellulase preparation used for producing the hypoallergenic flour, and the active compound was identified as Trp-Ser-Asn-Ser-Gly-Asn-Phe-Val-Gly-Gly-Lys.[3] Theoretically, it seems possible that the compound, which inhibits antigen absorption in the gastrointestinal tract, suppresses oral sensitization to the antigen. Therefore, we hypothesized that the enzyme-treated wheat flour containing the active peptide described above prevents oral sensitization to OVA. This study tested the hypothesis in animal models.

The animal experiments in this study were approved by the Hokkaido University Animal Use Committee, and animals were maintained in accordance with the guidelines for the care and use of laboratory animals at Hokkaido University.

Because Brown Norway rats have been reported to be a suitable model for oral sensitization to antigen protein,[4] we used this strain of rats to investigate the effects of enzyme-treated flour on oral sensitization to OVA. Three-week-old male Brown Norway rats were purchased from Japan Charles River (Tokyo, Japan). They were maintained in a temperature-controlled (23 ± 2°C) room with a dark period from 19:00 to 5:00 h, and acclimatized with free access to tap water and a purified diet prepared according to AIN-93G.[5] After 1 wk of acclimatization, rats were fed either an enzyme-treated flour diet or an untreated flour diet for 10 wk, and OVA solution (1 mg/ml, Sigma, MO, U.S.A.) was intragastrically administered to all animals every day. The enzyme-treated flour was prepared as previously described,[1] and the composition of the diets was also previously described.[2] Blood samples were obtained from the tail vein at weekly intervals and put through ELISA for measurement of OVA-specific IgG concentration as previously described.[2]

Figure 1 shows the time-course of changes in plasma levels of anti-OVA IgG after starting intragastric administration of OVA. In rats fed the untreated flour diet, an obvious increase of anti-OVA IgG was observed 2 wk after starting OVA administration, the levels reaching a plateau at 5 wk. In contrast, anti-OVA IgG levels in rats fed enzyme-treated flour began to increase at 3 wk and continued to increase gradually to the end of the experimental period. Plasma levels of anti-OVA IgG were consistently higher in rats fed the untreated flour diet than those fed the enzyme-treated flour diet throughout the experimental period, and there was a significant difference between the two groups at 4, 5, 6, and 7 wk. However, because anti-OVA IgG levels in rats fed the enzyme-treated flour diet almost caught up with those fed the untreated flour diet at the end of the experimental period, the data indicated that enzymatic modification of wheat flour in the diet

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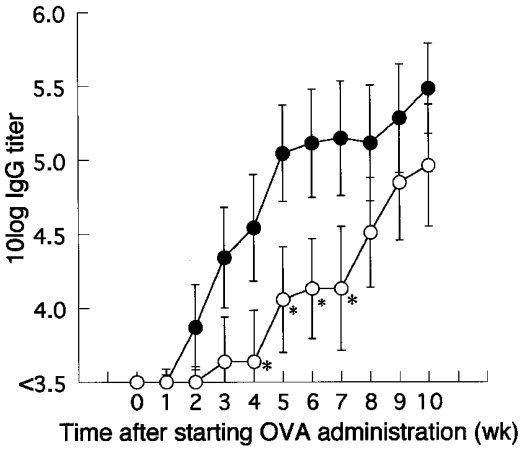

Fig. 1. Time-course of Changes in OVA-Specific IgG in Brown Norway Rats Daily Administered with OVA.

IgG titers were measured in plasma samples of rats obtained at weekly intervals. Open and closed circles represent enzyme-treated flour diet- and untreated flour diet-fed rats, respectively. Values are given as means ± SEM of 8–9 rats per group. An unpaired *t*-test was used to compare the mean values. $^{*}p<0.05$ compared with the untreated flour diet-fed group.

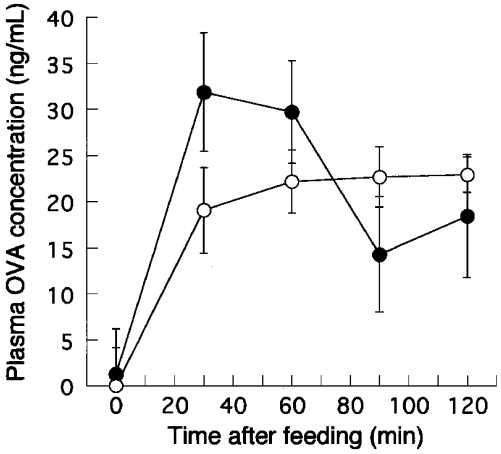

Fig. 2. Time-course of Changes in Plasma OVA Concentration after Feeding OVA-Supplemented Diets to BALB/c Mice.

Open and closed circles represent enzyme-treated flour diet- and untreated flour diet-fed mice, respectively. Values are given as means ± SEM of 10 mice per group.

retarded rather than prevented the sensitization to oral OVA. Because enzyme-treated flour contains the active peptide which would suppress intestinal absorption of OVA,[3] observed retardation of sensitization to oral OVA in rats fed enzyme-treated flour may be associated with suppression of antigen absorption. In order to examine this, the next experiment was done.

Five-week-old male BALB/c mice were purchased from Japan Charles River. They were maintained for 1 wk as described above. After withholding food for 15 h, we fed the mice 0.1 g of either enzyme-treated flour-containing diet or untreated flour-containing diet. The diets were supplemented with 20 mg/g of OVA. Blood samples were then collected from the retro-orbital vein at 0, 30, 60, 90, and 120 min after feeding, and the plasma OVA concentration was measured by sandwich ELISA. Microtiter plates (Becton Dickinson, NJ, U.S.A.) were coated with rabbit anti-OVA IgG (Chemicon, CA, U.S.A.) at 37°C for 2 h and then blocked with 1% bovine serum albumin (fraction V, Serologicals Proteins, IL, U.S.A.) at 37°C for 1 h. Diluted plasma samples were incubated in the well at 37°C for 2 h. After that, horseradish peroxidase-conjugated rabbit anti-OVA IgG (Rockland, PA, U.S.A.) was added and incubated at 37°C for 2 h. Between each pair of steps, the wells were washed with PBS containing 0.02% Tween-20. An enzyme substrate solution of 3,3′,5,5′-tetramethylbenzidine (Sigma) was used for color development. Finally, 2 mol/l H_2SO_4 was added, and the absorbance at 450 nm was measured with a microplate reader (Model 550, Bio-Rad, CA, U.S.A.).

Figure 2 shows the time-course of changes in the plasma concentration of OVA after feeding the OVA-supplemented diets in mice. All mice consumed the diets within 5 min after it was given to them. The plasma concentration of OVA in mice fed untreated flour diet was highest at 30 min post feeding and decreased thereafter. In contrast, enzyme-treated flour diet-fed mice showed relatively constant levels of plasma OVA. Plasma OVA levels tended to be lower in mice fed the enzyme-treated flour diet than those fed the untreated flour diet at 30 and 60 min post feeding. The data suggest that enzyme-treated flour suppresses intestinal absorption of OVA, even though it remains unclear whether the previously identified active peptide is responsible for the suppression of OVA absorption. Other possibilities should be also considered, since enzymatic modification of wheat flour may produce some compounds which increase the degradation of OVA, potentiate the secretion of IgA eliminating OVA, and/or strengthen the nonspecific mucosal barrier.

Together, these findings suggest that enzyme-treated flour retards oral sensitization to a typical food allergen, OVA, and that the suppressive effect of enzyme-treated flour on OVA absorption may be relevant to retarded sensitization observed in rats. Because antigen permeability through the intestinal tract has been reported to be increased in allergic patients,[6–8] suppression of antigen absorption in the intestine would be a promising approach to prevent not only the sensitization to food antigens in healthy people but also the allergic inflammation in allergic patients. Therefore we propose that enzyme-treated flour is applicable to prevent food allergy.

Acknowledgments

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