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Oral Tolerance Is Not Influenced by Oral Application of Oil-Emulsified Proteins

Key Words

Food allergy
Oral tolerance
Emulsion
Prophylaxis

Abstract

A 2-week program of feeding protein antigens as an oil-in-water emulsion to naive mice elicited a significant serum IgG antibody response, whereas their aqueous preparations did not at all. The unresponsive immune state that had been developed after feeding aqueous antigen was not disturbed by subsequent oral challenge with the same antigen in the presence of oil. These results suggest that the principle of oral tolerance is a feasible strategy for prophylaxis of hypersensitization to protein antigens, where protein tolerogens, in this case, are to be given without any additives at their first introduction.

As a contradictory phenomenon to the incidence of food allergies in humans, it has been convincingly demonstrated in experimental animals that feeding of protein antigens is inefficient in generating antibody responses [1–3]. Taking into account the different regimen accompanied with the inconsistent immune responses to dietary antigens between humans and animals, it might be relevant to consider that the response to a single purified food component can be different from the one to the whole food. We investigated if feeding oil that was emulsified with protein antigen can evoke a humoral immune response to the antigen therein and evaluated the prophylactic significance of oral pretreatment with an antigen free of oil in the sensitization to the same antigen in emulsions subsequently ingested.

BALB/c mice were intragastrically given 10 doses of 25-mg bovine β -lactoglobulin (β -Lg) dissolved in distilled water or oil-in-water emulsion of 50 μ l soybean oil over the period of 12 days. A remarkable increase in anti- β -Lg IgG antibody was generated in sera from mice which received the emulsion compared to no increase at all in mice receiving the aqueous solution (fig. 1). The adjuvant-like effect of oil in emulsion was also demonstrated when human

(HSA) and bovine (BSA) serum albumin-stabilized emulsions were fed, suggesting that oil in food may play an important role for the sensitivity to protein antigens therein.

A cross-feeding study was conducted to see if the unresponsive immune state developed after frequent feeding of aqueous antigen was disturbed by the subsequent oral challenge with the same antigen in the presence of oil. It was found that the 2-week oral challenge with β -Lg-stabilized emulsion failed to generate specific IgG antibodies when mice had been orally treated with aqueous β -Lg in advance (fig. 2). On the other hand, feeding aqueous β -Lg for 3 days enhanced the ongoing antibody production.

Until now the immunological mechanisms underlying the induction of antibody response after feeding protein antigens in an oil-in-water emulsion have remained unclear. However, results obtained using a newly developed oral immunization model encourage us to propose that the peroral treatment with protein antigens as tolerogens is a feasible strategy for prophylaxis of sensitization to those in food, where purified proteins are to be given without any additives at their first introduction.

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Fig. 1. Serum IgG antibody responses following oral immunization with protein antigens. Mice were fed 10 doses of 25 mg/head β -Lg, HSA or BSA in the presence (right column) or absence (left column) of 50 μ l soybean oil emulsified with the respective antigens on days 0–4 and 7–11. Sera taken on the indicated day (diluted to 1:50) were analyzed for IgG antibody specific for fed antigen by means of ELISA. The results presented are means $\pm$ SE for 5 mice per group. ** $p < 0.01$ compared with mice fed the corresponding aqueous preparation using the Mann-Whitney U test.

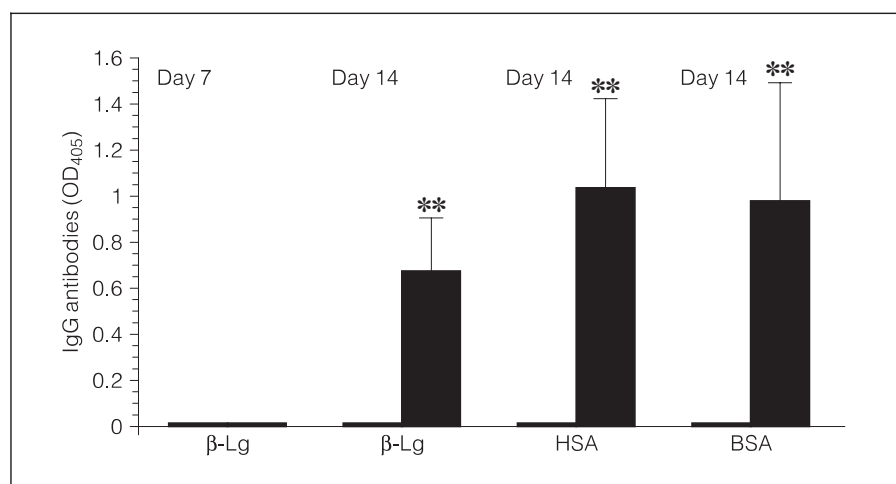
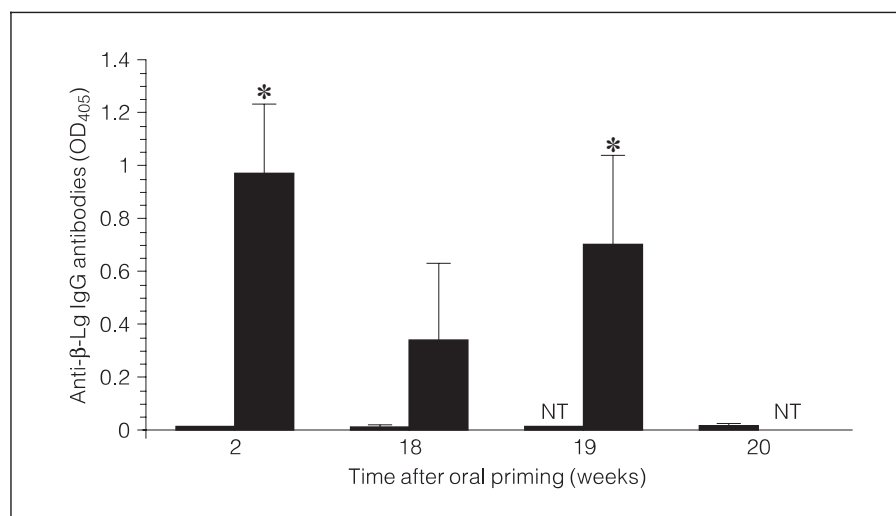


Fig. 2. Serum IgG antibody responses in a cross-over feeding study. Mice were orally primed with β -Lg (10 doses) in the presence (right column) or absence (left column) of oil as described in figure 1. Both groups of mice were bled 3 days (2 weeks) and 4 months later (18 weeks). Then, the emulsion-primed mice were orally challenged with 3 doses of oil-free β -Lg solution for 3 successive days and bled 5 days later (19 weeks), while the oil-free β -Lg-primed mice were challenged with 10 doses of β -Lg-stabilized emulsion and bled 3 days later (20 weeks). Data presented are means $\pm$ SE for 5 mice per group. * $p < 0.05$ compared with mice at 18 weeks using the Wilcoxon test. NT Not tested.



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