



Note

Oral Administration of Alginic Acid Oligosaccharide Suppresses IgE Production and Inhibits the Induction of Oral Tolerance

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We have found that alginic acid oligosaccharide (ALGO) enhanced Th1 by promoting IL-12 production, suggesting that ALGO can be applied as an anti-allergic food. In this study we examined both positive and negative functions of ALGO. First we investigated the anti-allergic activity of ALGO, as a positive function, when orally administered. IgE production was significantly inhibited in mice fed ALGO as compared to control mice. This result indicates that ALGO had anti-allergic activity even when orally administered. On the other hand, we also found a negative function of ALGO. Oral co-administration of a protein antigen and ALGO inhibited the induction of oral tolerance to the protein. These data indicate the potential of ALGO as an anti-allergic food material and the necessity of further examination to determine a safe method application.

Key words: alginic acid oligosaccharide; anti-allergic food; IgE; IL-12; oral tolerance

The type I allergic reaction is mediated by the allergen-specific IgE antibody. The development of a method for inhibiting IgE production should therefore be a useful approach to preventing allergic disorders. In particular, IgE-inhibiting food materials can be expected to control allergic diseases through everyday meals. Several food materials have so far been studied and demonstrated to be anti-allergic.¹⁻³⁾ We have found that alginic acid oligosaccharide (ALGO), which is a lyase lysate of alginic acid with an average degree of polymerization of 4.4, inhibited IgE production in a mouse model.⁴⁾ We also found that this function might be mediated by the IL-12-enhancing activity of ALGO, but in that study we examined only the IgE-inhibiting activity of ALGO when it was intraperitoneally administered. We considered that it would be necessary to test the activity of ALGO when it was orally administered to apply ALGO as an anti-allergic food material.

In this present study we investigated the effect of

orally administered ALGO on IgE production. Female BALB/c mice (6 weeks old; Clea Japan, Tokyo) were given 10 mg of ALGO dissolved in 200 μ l of PBS five times by feeding needle. Each feed was given at an interval of 2-3 d. These mice were intraperitoneally immunized with 100 μ g of bovine β -lactoglobulin (β -LG, genotype AA) mixed with 2 mg of an alum adjuvant after the second feed of ALGO. Two weeks after immunization, the mice were bled. The blood samples were centrifuged and the sera were collected. The β -LG-specific IgE titer in the sera was measured by ELISA. Maxisorp immunoplates (Nunc, Roskilde, Denmark) were coated with a 0.01% β -LG solution. After washing and blocking, the sample sera and standards were added to the plate. The bound antibody was detected with biotin-labeled rat anti-mouse IgE (LO-ME-2; Technopharm Biotechnology, Villejuif, France), before incubating with alkaline phosphatase-streptavidine. A substrate (*p*-nitrophenyl-phosphate) was then added, and the absorbance was determined at 405 nm. Student's *t*-test was used to analyze the statistical difference, a *P* value of less than 0.05 being considered statistically significant. As shown in Fig. 1, IgE production by the ALGO-fed mice was significantly lower than that by the PBS-fed control mice. IgG1 production by the ALGO-fed mice also showed a tendency to be inhibited, although the inhibition was not statistically significant (data not shown). On the other hand, IgG2a production by the ALGO-fed mice was similar to that by the control mice (data not shown). We considered that the induction of IgG2a might be enhanced by the Th1-inducing ability of ALGO, but it was erased by the IL-4-inhibiting ability of ALGO, because IL-4 is necessary to produce not only IgE and IgG1 but also other isotypes. These results indicate that orally administered ALGO suppressed IgE production, demonstrating the potential for ALGO to be used as an anti-allergic food material. This effect of ALGO was not apparent when we administered 1 mg of ALGO five times (data not shown), indicating that

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Abbreviations: ALGO, alginic acid oligosaccharide; β -LG, β -lactoglobulin; LAB, lactic acid bacterium

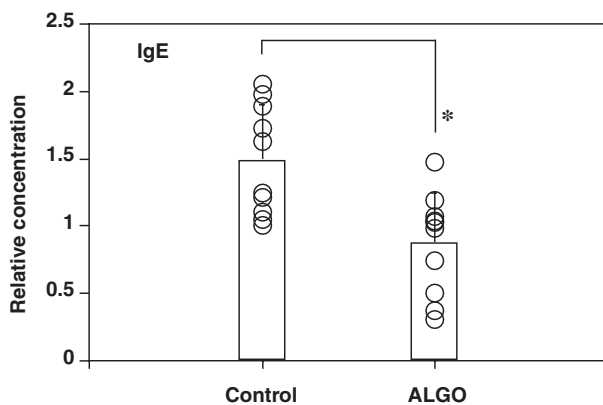


Fig. 1. Effect of Orally Administered ALGO on IgE Production.

BALB/c mice were fed five times with 10 mg each of ALGO. The mice were immunized i.p. with β -LG mixed with an alum adjuvant after the second feed. Two weeks after immunization, the mice were bled and sera samples were prepared. The titer of β -LG-specific IgE in the sample sera was calculated relative to that of β -LG-specific IgE in the standard serum. The β -LG-specific IgE titer in the sera of mice fed ALGO was significantly lower than that of the control mice. These results are representative of two independent experiments. *Significantly different from the control value ($p < 0.05$).

10 mg of ALGO five times is almost the lowest amount necessary to inhibit IgE production.

Several previous studies have reported that some strains of lactic acid bacteria (LAB) induced a high level of IL-12 production and inhibited IgE production.⁵⁻⁷ Some of these studies also showed that LAB retained this activity when orally administered.^{6,7} This activity is similar to that of ALGO. However, the mechanism by which orally administered LAB or ALGO inhibited IgE production has not been determined; for example, the sites of operation. We consider that ALGO has an advantage for working *in vivo* as compared to LAB when administered orally, because ALGO is water soluble and might easily be absorbed and transported to several sites where IgE is induced. We have found that the activity of ALGO to inhibit IgE production increased with increasing degree of polymerization of ALGO,⁴ but highly polymerized ALGO is insoluble and forms a gel in the presence of Ca^{2+} or Mg^{2+} . A study is now in progress in our laboratory to investigate the most effective degree of polymerization of ALGO for inhibiting IgE production when administered orally.

Oral tolerance is an immunologically unresponsive state to an orally administered antigen. This mechanism represents the major immune response in the intestines, and has been considered to be critical for preventing food allergy.⁸ We have reported that oral administration of IL-12 abrogated the induction of oral tolerance to an antigen fed simultaneously with IL-12.⁹ This evidence indicates that not only IL-12 but also some food factors abrogated the induction of oral tolerance, suggesting that such food factors are a trigger for hypersensitivity to food antigens. If ALGO also inhibits the induction of oral tolerance, that is a negative indication for applying

it as an anti-allergic food. We must examine the effect on oral tolerance of such food factors as ALGO and LAB which induce IL-12.

Hence we investigated the effect of orally co-administering the antigen and ALGO on the induction of oral tolerance. BALB/c mice were given 200 μg of β -LG and 10 mg of ALGO dissolved in 200 μl of PBS four times at an interval of 2–3 d. This amount of ALGO, 10 mg/feed, was the same as that used in the IgE inhibiting experiment, and almost the lowest amount necessary to inhibit IgE production. β -LG (100 μg) was emulsified in Freund's complete adjuvant, and the mice were intraperitoneally immunized 2 d after the last feed. Two weeks after this immunization, 100 μg of β -LG was emulsified in Freund's incomplete adjuvant and the mice were boosted intraperitoneally. The mice were bled 10 d after boosting and sera samples were prepared. The β -LG-specific antibody titer in the sera was measured by ELISA. Biotin-labeled rat anti-mouse IgG1 (ZYMED, San Francisco, CA) was used for detection of IgG1, and alkaline phosphatase-labeled rabbit anti-mouse immunoglobulins (Dako Cytomation, Glostrup, Denmark) was used for detection of total Ig. As shown in Fig. 2, the levels of β -LG-specific IgG1 and total Ig of the mice fed β -LG alone were both significantly lower than those of the PBS-fed control mice, indicating induction of oral tolerance. Although the mice fed both β -LG and ALGO also showed a significantly lower antibody titer than the control animals, the titer level was significantly higher than that of the mice fed β -LG alone. These data indicate that oral administration of ALGO with a protein antigen inhibited the induction of oral tolerance to the antigen.

We have reported that oral administration of IL-12 inhibited the induction of oral tolerance to the protein antigen fed with IL-12.⁹ We considered that ALGO would also affect oral tolerance through IL-12, because IL-12 is a critical factor induced by ALGO in inhibiting IgE production.⁴ Our results in this study suggest strongly that some food factors which induce IL-12 and can be expected to have anti-allergic, anti-tumor, and anti-virus activity would inhibit the induction of oral tolerance. This observation suggests some risk from such food factors, because the abrogation of oral tolerance would cause hyper-responsiveness to food antigens, leading to inflammation of the intestines and/or food allergy.⁸ This appears to be a negative indication for ALGO. On the other hand, the results of our previous study showed that oral administration of IL-12 abrogated only the induction of oral tolerance, and not the maintenance of it.⁹ Furthermore, we found that oral tolerance can be induced normally in mice that have failed to induce such tolerance by simultaneous feeding of the antigen and IL-12.⁹ We conclude from all this evidence that ALGO can be used as a novel anti-allergic food material, although it also has a negative function of inhibiting the induction of oral tolerance, because this risk is very low.

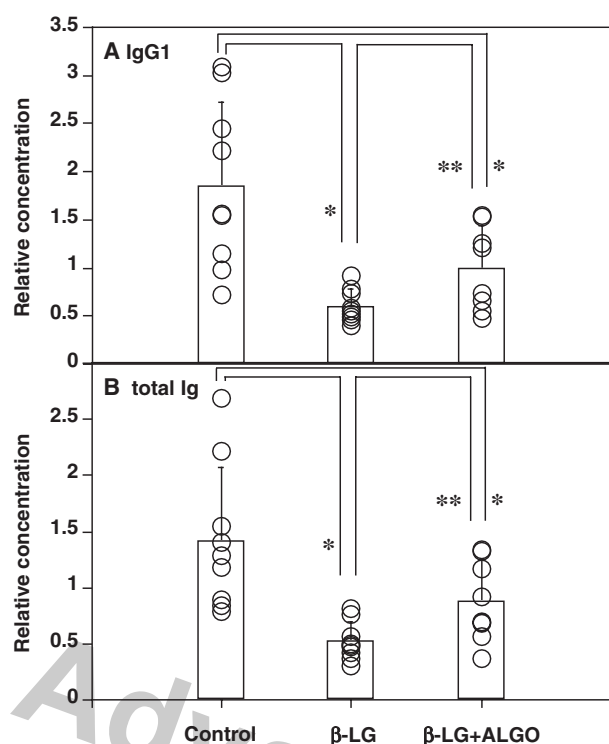


Fig. 2. Effect of Orally Administered ALGO on the Induction of Oral Tolerance.

BALB/c mice were simultaneously fed four times with 10 mg each of ALGO and 200 μ g each of β -LG. The mice were immunized i.p. with β -LG emulsified in Freund's complete adjuvant after the last feed. Two weeks after immunization, the mice were boosted i.p. with the antigen emulsified in Freund's incomplete adjuvant. Two weeks later, they were bled and sera samples were prepared. The titers of β -LG-specific IgG1 and total Ig in the sample sera were calculated relative to those of β -LG-specific IgG1 and total Ig in the standard serum. The β -LG-specific IgG1 (A) and total Ig (B) titers in the sera of the mice fed β -LG alone were significantly lower than those of the control mice. On the other hand, the β -LG-specific IgG1 and total Ig titers in the sera of the mice fed both β -LG and ALGO were significantly higher than those of the mice fed β -LG alone, although significantly lower than those of the control mice. These results are representative of two independent experiments. *Significantly different from the control value ($p < 0.05$). **Significantly different from the value of mice fed β -LG only ($p < 0.05$).

Our results indicate that food factors that induce IL-12 abrogate the induction of oral tolerance to an antigen fed with the factors. On the other hand, Tsuji *et al.* have reported that λ -carrageenan, also a food factor inducing IL-12, did not affect the induction of oral tolerance.¹⁰ This evidence suggests that λ -carrageenan might enhance the induction of oral tolerance, and that such activity might work to erase the effect of IL-12. They have also reported that λ -carrageenan enhanced the induction of oral tolerance in C3H/HeJ mice that express impaired TLR-4. This result strongly supports the hypothesis mentioned above. We also found that ALGO inhibited the induction of oral tolerance, although this inhibition was incomplete. This result might indicate that ALGO inhibited antibody produc-

tion, because ALGO inhibits IL-4 production and IL-4 is important in antibody production. On the other hand, it might indicate that ALGO has ability both to inhibit and to enhance oral tolerance similarly to λ -carrageenan, or that the amount of administered ALGO was insufficient for complete inhibition. Further investigation is needed to reveal the enhancing mechanism for oral tolerance by food factors and to understand better the particular influence of each food factor on oral tolerance.

The aim of this study was to examine the effect of orally administered ALGO on the production of IgE and the induction of oral tolerance, the same amount of ALGO being used for the test on IgE production and oral tolerance. All mice were maintained and used in accordance with the guidelines for the care and use of experimental animals of Tokyo University of Agriculture and Technology. The results strongly indicate both positive and negative functions of ALGO. ALGO was efficient as an anti-allergic food material, while it had the side effect of inhibiting the induction of oral tolerance. The evidence obtained in this study can be applied to other food factors that induce IL-12, such as some strains of LAB and some kinds of mushroom.

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