

Alginate Acid Oligosaccharide Suppresses Th2 Development and IgE Production by Inducing IL-12 Production

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Key Words

Alginate acid oligosaccharide · IgE · IL-12 · Th1/Th2

Abstract

Background: Since allergen-specific IgE is directly involved in the type I allergic reaction, development of a method for inhibiting Th2 responses which lead to the induction of IgE production would be a useful approach for preventing allergic disorders. The ability and mechanism of alginate acid oligosaccharide (ALGO), an oligosaccharide obtained from natural edible polysaccharide, for suppressing Th2 responses was examined in detail. **Methods:** Lymph node cells obtained from β -lactoglobulin (β -LG)-primed BALB/c mice were cultured in vitro with an antigen for 3 days in the absence or presence of ALGO. The amount of cytokine in each culture supernatant was measured. The effect of ALGO on Th2 development was also examined by using ovalbumin specific T cell receptor transgenic mice. Antibody production in the serum of BALB/c mice that had been immunized with β -LG or β -LG plus ALGO was investigated. **Results:** The production of IFN- γ induced by antigen stimulation was upregulated by ALGO in a dose-dependent manner. IL-12 production was also enhanced by ALGO, and the

addition of the anti-IL-12 antibody to the culture abrogated the effect of ALGO. On the other hand, IL-4 production by antigen-stimulated splenocytes of transgenic mice was suppressed in the presence of ALGO. Furthermore, IgE production by ALGO-treated mice was significantly inhibited compared with control mice. **Conclusions:** These results indicate that ALGO suppressed antigen-induced Th2 development by inducing IL-12 production. ALGO also inhibited in vivo IgE production. These findings suggest that ALGO is expected to be an edible anti-allergic agent.

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Introduction

The type I allergic reaction against such agents as house dust, pollen and food is mediated by the allergen-specific IgE antibody. The development of a method for inhibiting IgE production would therefore be a useful approach for preventing allergic disorders. It is known that the balance of Th1- and Th2-type cytokines regulates the induction of IgE. Th2 secretes IL-4, which is a critical cytokine for inducing IgE, while Th1 produces IFN- γ [1]. Since IFN- γ suppresses Th2 differentiation and/or expansion

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sion [2], and isotype switching of B cells to IgE-producing cells [3], potentiating the Th1 response would be useful for controlling allergic diseases. Shida et al. [4, 5] have reported that *Lactobacillus casei*, a member of the lactic acid bacteria family, inhibited antigen-induced IgE production in a murine model in vitro and in vivo. Tsuji et al. [6] have revealed that λ -carrageenan suppresses antigen-induced IgE production and serum histamine release in mice. Shibata et al. [7] have demonstrated that chitin particles induce IL-12 production, and downregulated the IgE level and lung eosinophilia in allergic mice. Shirota et al. [8] have shown that CpG oligodeoxynucleotides conjugated with antigen regulated murine airway eosinophilia.

Alginic acid is a natural polysaccharide, and numerous physiological functions of alginic acid and its lyase-lysate, alginate oligosaccharide (ALGO), have been reported [9–11]. In addition, Fujihara and Nagumo [12] have revealed the anti-tumor activity of alginic acid and Kawada et al. [13, 14] have reported that ALGO enhances the growth of human endothelial cells [13] and keratinocytes [14]. Although the results of our previous studies indicate that ALGO affected some immune responses [unpublished observation], the effect of ALGO on the Th1 and Th2 balance has not been clarified. It could be expected that ALGO would suppress the Th2 response and IgE production by enhancing the Th1 response which would lead to inhibiting the allergic reaction.

The mechanism by which ALGO controls the Th1 and Th2 balance must be investigated in order to use ALGO to humans. It has been reported from previous studies that the recognition of many Th1 potentiators was mediated by a pattern recognition receptor (PRR). Chitin particles have been reported to be recognized by the mannose receptor [15], and lipopolysaccharide (LPS) of gram-negative bacteria is known to be recognized by toll-like receptor (TLR) 4 [16]. In addition to these agents, it has recently been demonstrated that many bacterial components were recognized by TLRs [17, 18]. It is considered that ALGO may also be recognized by a PRR and affect the balance of Th1 and Th2.

In this study, we examined the activity of ALGO and its mechanism for enhancing Th1 and inhibiting Th2 responses in detail. Our results demonstrate that ALGO interacted with non-T and non-B cells, perhaps macrophages or dendritic cells (DC), to induce IL-12 production by those cells and enhance Th1 development. Furthermore, ALGO could suppress Th2 development and IgE production. Since alginic acid is widely used as a food material, ALGO should be a novel anti-allergic agent not causing any harm to humans.

Materials and Methods

Mice

Female BALB/c, C3H/HeN and C3H/HeJ mice (6 weeks old) were purchased from Clea Japan (Tokyo, Japan) and used at 6–9 weeks of age. Male ovalbumin (OVA)-specific T cell receptor (TCR) transgenic mice (DO11.10) were used at 9–12 weeks of age [19]. T cells of these mice recognize OVA_{323–339} restricted to I-A^d. 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.

Antigen

Crude bovine β -lactoglobulin (β -LG; genotype AA) was prepared from fresh milk of a Holstein cow according to the method of Armstrong et al. [20]. Crude β -LG was purified with a DEAE-Sepharose Fast Flow column (2.5 ID $\times$ 50 cm; Amersham Pharmacia Biotech, Buckinghamshire, UK) by the method described previously [21]. The purity of β -LG was confirmed by polyacrylamide gel electrophoresis (PAGE), performed by the method of Davis [22]. OVA was purchased from Seikagaku Kogyo (Tokyo, Japan).

ALGO

ALGO [the lyase-lysate of alginic acid; average degree of polymerization (DP) = 4.4 and the average molecular weight = 713] was kindly provided by Meiji Seika Co. Ltd. (Tokyo, Japan). LPS was removed from ALGO with AffinityPak Detoxi-Gel endotoxin-removing gel (Pierce, Rockford, Ill., USA). ALGO was fractionated in a gel filtration column (Ampure SA code RPN. 1754; Amersham Pharmacia Biotech) into fractions A, B, C and D in decreasing molecular weights. The respective DP values of the fractions were determined by size exclusion chromatography as 5.2, 4.5, 4.2 and 3.7, and the average molecular weight of those were 842, 729, 680 and 599.

Immunization

The in vitro lymph node cell culture used BALB/c or C3H mice that had been immunized subcutaneously with 100 μ g of β -LG emulsified in Freund's complete adjuvant (CFA) H37Ra (Difco Laboratories, Detroit, Mich., USA). Ten days after immunization, the inguinal and popliteal lymph nodes were obtained from the sensitized mice, and single-cell suspensions were prepared. The in vivo antibody production assay used BALB/c mice that had been immunized intraperitoneally with 50 μ g of β -LG alone or with β -LG plus 200 μ g of ALGO absorbed to an alum adjuvant (prepared in our laboratory) three times every 2 days. These mice were bled 4 weeks after the first immunization. The blood samples were centrifuged, and the sera were collected and stored at -30°C until needed.

Cell Culture

The lymph node cells obtained from the β -LG-immunized BALB/c and C3H mice, and the erythrocyte-depleted splenocytes of untreated TCR transgenic mice were cultured at 2×10^6 cells/ml in the presence of 1 or 10 μM β -LG, or 0.2 or 2 μM OVA in RPMI-1640 (Nissui Pharmaceutical, Tokyo, Japan) containing 10% fetal calf serum (JRH Biosciences, Lenexa, Kans., USA) or 1% normal mouse serum. Three to five mice were used in each experiment. The cells obtained from individual mice were pooled and used for the experiments. ALGO (18.4 or 184 $\mu\text{g/ml}$) was added to these cultures. The anti-IL-12 antibody (C17.8; Genzyme, Cambridge, Mass., USA) was also added to the culture in some experiments. Three days later, each

culture supernatant was collected, and the amount of cytokines in the supernatant was measured. The lymph node cells of the β -LG-immunized BALB/c mice were used after fractionation into T cells, non-T cells, B cells or non-B cells with an MACS system (Miltenyi Biotec, Bergish Gladbach, Germany) in some experiments. Anti-mouse CD90 and anti-mouse CD45R microbeads were used for selecting or depleting the T cells and B cells, respectively. The lymph node cells from 10 mice were pooled and used for these experiments.

Cytokine Detection

The concentration of cytokines in each culture supernatant was assessed using a sandwich ELISA. For determination of IL-12 p70, a Ready-SET-Go ELISA kit (eBioscience, San Diego, Calif., USA) was used. For detection of IFN- γ and IL-4, Maxisorp immunoplates (Nunc, Roskilde, Denmark) were coated with the rat anti-mouse IFN- γ (R4-6A2; BD Pharmingen, San Diego, Calif., USA) or rat anti-mouse IL-4 antibody (11B11; BD Pharmingen). After washing and blocking, the culture supernatant and standards were added to the plates. Bound cytokine was detected with the biotin-labeled monoclonal rat anti-mouse IFN- γ (XMG1.2; BD Pharmingen) or rat anti-mouse IL-4 antibody (BVD6-24G2; BD Pharmingen), before incubation with alkaline phosphatase-streptavidine (Zymed, San Francisco, Calif., USA). A substrate (*p*-nitrophenyl-phosphate) was added, and the absorbance was determined at 405 nm.

Serum Antibody Detection

β -LG-specific antibody titers in the sera were measured by ELISA. Maxisorp immunoplates were coated with a 0.01% β -LG solution. After washing and blocking, the sample sera and standards were added to the plates. The bound antibody was detected with the biotin-labeled rat anti-mouse IgE (LO-ME-3; Serotec, Oxford, UK), biotin-labeled rabbit anti-mouse IgG1 or biotin-labeled rabbit anti-mouse IgG2a (Zymed), before incubation with alkaline phosphatase-streptavidine. A substrate (*p*-nitrophenyl-phosphate) was added, and the absorbance was determined at 405 nm.

Statistical Analysis

Differences between the control and ALGO-treated groups were analyzed by Student's *t* test. *p* < 0.05 is considered statistically significant.

Results

ALGO Upregulates IFN- γ and IL-12 Production by Lymph Node Cells

The effect of ALGO on the cytokine production pattern of cultured lymph node cells was tested *in vitro*. The level of IFN- γ production by lymph node cells obtained from the β -LG-immunized BALB/c mice was measured. As shown in figure 1, ALGO upregulated the antigen-induced IFN- γ production in a dose-dependent manner (fig. 1a). LPS-free ALGO also enhanced the IFN- γ production (fig. 1b). The activity of ALGO was dependent on the molecular weight, highly polymerized ALGO (fraction A, fig. 2a; fraction B, fig. 2b) showing stronger activity

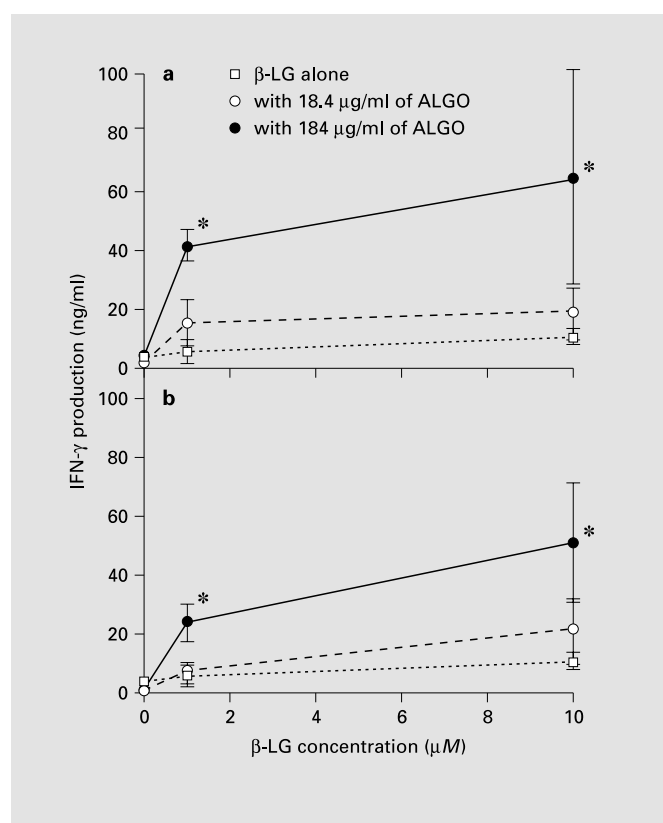


Fig. 1. Effect of ALGO on β -LG-induced IFN- γ production by BALB/c lymph node cells. Lymph node cells from β -LG-primed BALB/c mice were cultured with β -LG in the absence (control), or presence of untreated (a) or LPS-free (b) ALGO. The amount of IFN- γ in each supernatant on day 3 was determined by ELISA. The result is representative of three independent experiments. * Significant difference compared with the control.

than low-molecular-weight ALGO (fraction C, fig. 2c; fraction D, fig. 2d).

IL-12 is known as a potent and general inducer of Th1 differentiation [23]. Table 1 shows that ALGO strongly upregulated IL-12 production by lymph node cells, this upregulation of IL-12 production being independent of the antigen. Furthermore, the IFN- γ -enhancing activity of ALGO was completely abrogated by blocking IL-12 with the anti-IL-12 antibody. As shown in figure 3, IFN- γ production was inhibited by adding the anti-IL-12 antibody to the same level as the control that was cultured in the absence of ALGO. These results indicate that ALGO affected IFN- γ production by enhancing IL-12 production.

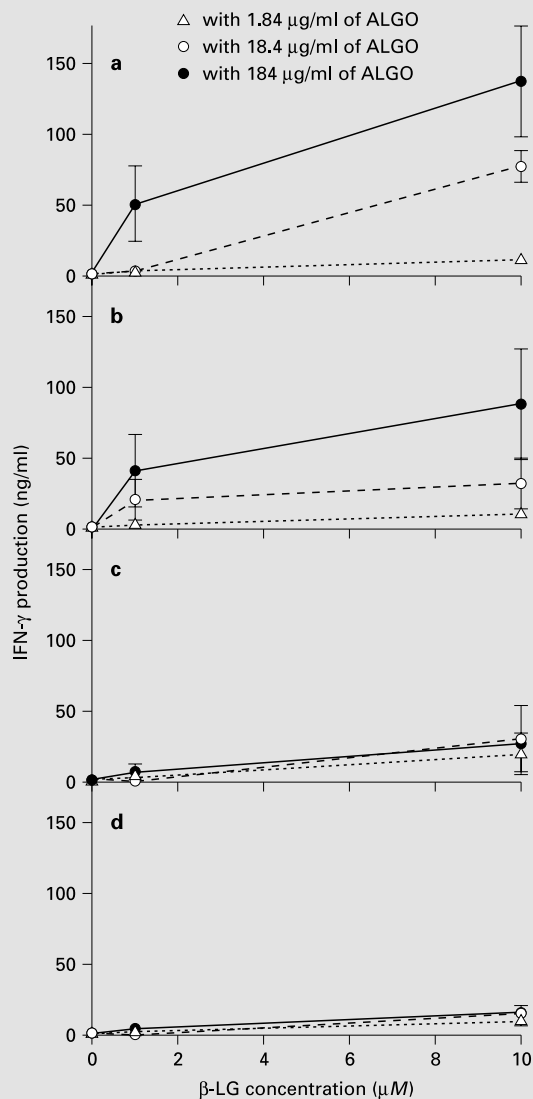


Fig. 2. Effect of fractionated ALGO on β -LG-induced IFN- γ production. Experiments similar to those described in figure 1 were performed. Lymph node cells were cultured with fraction A (**a**), fraction B (**b**), fraction C (**c**) or fraction D (**d**). The result is representative of two independent experiments.

Non-B Cells but Not B Cells Were Required for Enhancing IFN- γ Production by ALGO

It seemed that ALGO affected T cells to enhance IFN- γ production by interacting with other cell populations, because the effect of ALGO was mediated by the upregulation of IL-12 production. To investigate which cell pop-

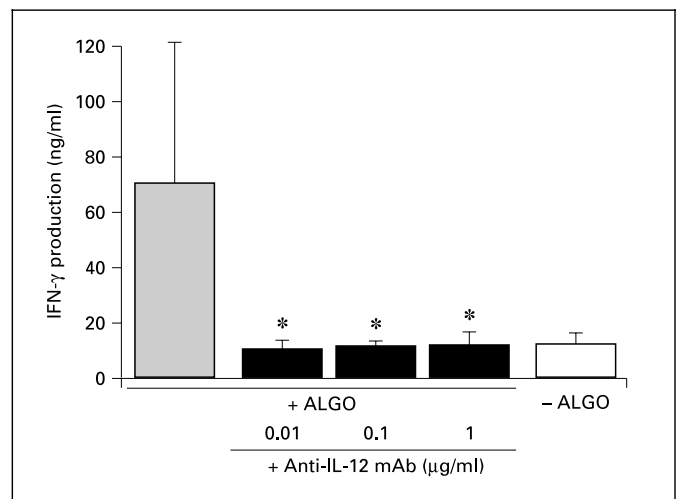


Fig. 3. Effect of anti-IL-12 antibody on the ALGO-mediated upregulation of IFN- γ production. Lymph node cells from β -LG-primed BALB/c mice were cultured with β -LG (5 μ M) in the absence or presence of ALGO (18.2 μ g/ml). The anti-IL-12 antibody (0.01–1 μ g/ml) was added to the culture throughout the culture period. The amount of IFN- γ in each supernatant on day 3 was determined by ELISA. The result is representative of two independent experiments. * Significant difference compared with the samples cultured in the absence of antibody.

Table 1. Effect of ALGO on IL-12 production by lymph node cells

β -LG concentration μ M	IL-12 production, pg/ml	
	no ALGO	with ALGO
0	23.0 $\pm$ 5.6	89.9 $\pm$ 6.4*
1	30.1 $\pm$ 5.7	70.1 $\pm$ 4.9*
5	32.5 $\pm$ 3.1	70.8 $\pm$ 3.4*
10	23.9 $\pm$ 2.5	66.7 $\pm$ 5.4*

Lymph node cells obtained from β -LG-primed BALB/c mice were cultured with β -LG in the absence or presence of ALGO (184 μ g/ml). The level of IL-12 in each supernatant on day 3 was measured by ELISA. Each result is shown as the mean $\pm$ SD and is representative of two independent experiments.

* Significant difference compared with the control.

ulations directly responded to ALGO, the response to ALGO of the fractionated cells was examined after the T cells and B cells had been isolated or depleted from the lymph node cells. Culture of the T cells alone, non-T cells or B cells alone produced hardly any IFN- γ either with or without β -LG and ALGO (data not shown). This evidence

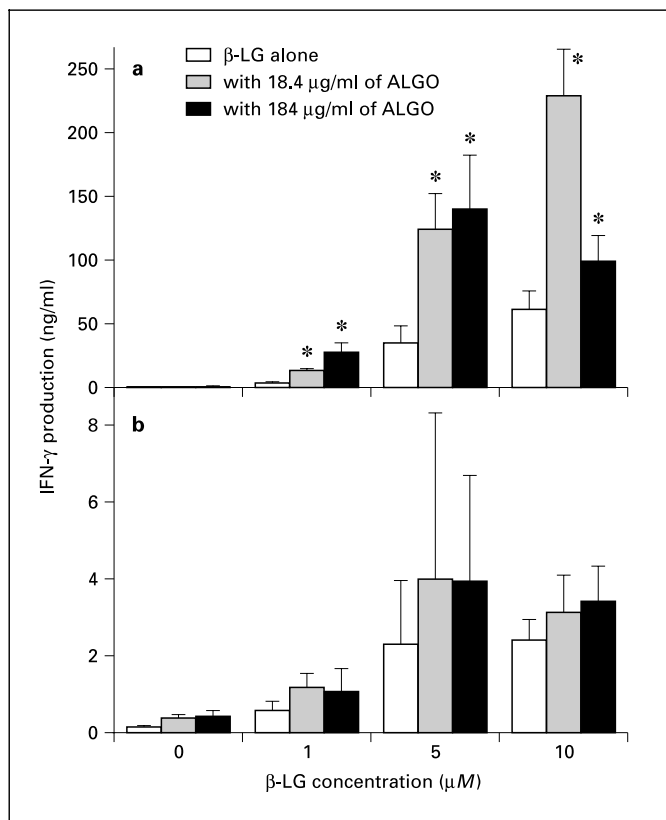


Fig. 4. Effect of ALGO on β -LG-induced IFN- γ production by isolated lymph node cells. Lymph node cells from β -LG-primed BALB/c mice were fractionated with MACS as described in the Materials and Methods section. The non-B cell fraction was used as described for figure 1 (**a**). Purified T cells and B cells were mixed at 1×10^6 cells/ml each (**b**). The amount of IFN- γ on day 3 in each supernatant was determined by ELISA. The result is representative of three independent experiments. * Significant difference compared with the control.

confirms that IFN- γ was mainly secreted by antigen-specific T cells in this study, and that ALGO did not directly affect the T cells to induce IFN- γ production. The culture of non-B cells was significantly affected by ALGO in the presence of β -LG (fig. 4a). On the other hand, although the mixed culture of T cells and B cells produced a small amount of IFN- γ in response to the antigen, the effect of ALGO was minimal (fig. 4b). These results demonstrate that ALGO interacted with non-T and non-B cells, perhaps macrophages or DC.

ALGO Affected Lymph Node Cells via a Mechanism Other than TLR4

Our findings revealed that ALGO upregulated IFN- γ production by enhancing IL-12 production from macro-

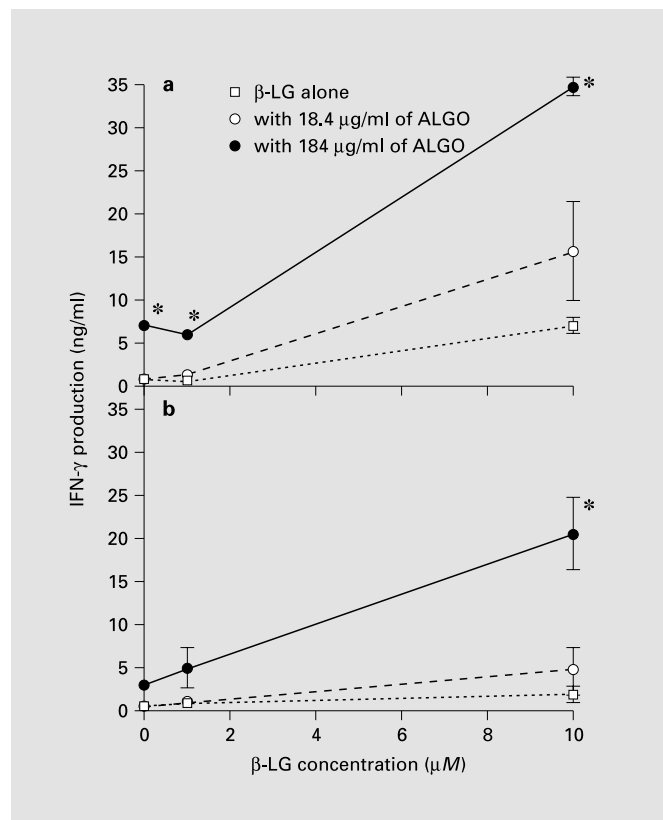


Fig. 5. Effect of ALGO on β -LG-induced IFN- γ production by C3H lymph node cells. Lymph node cells from β -LG-primed C3H/HeN (**a**) and C3H/HeJ (**b**) mice were cultured and tested according to the method described for the experiment in figure 1. The result is representative of three independent experiments.

phages or DC. It is known that these innate immune cells respond to LPS via TLR4 [16]. LPS is a membrane component of gram-negative bacteria and a strong potentiator of IL-12. We next examined the involvement of TLR4 in the response to ALGO. C3H/HeJ mice were used, because these mice cannot respond to LPS due to the lack of their TLR4 function [24]. C3H/HeN mice, which can respond to LPS, were used as controls. ALGO enhanced the IFN- γ production by lymph node cells of both C3H/HeN mice (fig. 5a) and C3H/HeJ mice (fig. 5b). On the other hand, the amount of IFN- γ produced by the cells of C3H/HeJ mice stimulated with ALGO was lower than that of C3H/HeN mice. We considered that this did not show a lower effect of ALGO in C3H/HeJ mice but a weaker response to β -LG, because IFN- γ production induced by β -LG

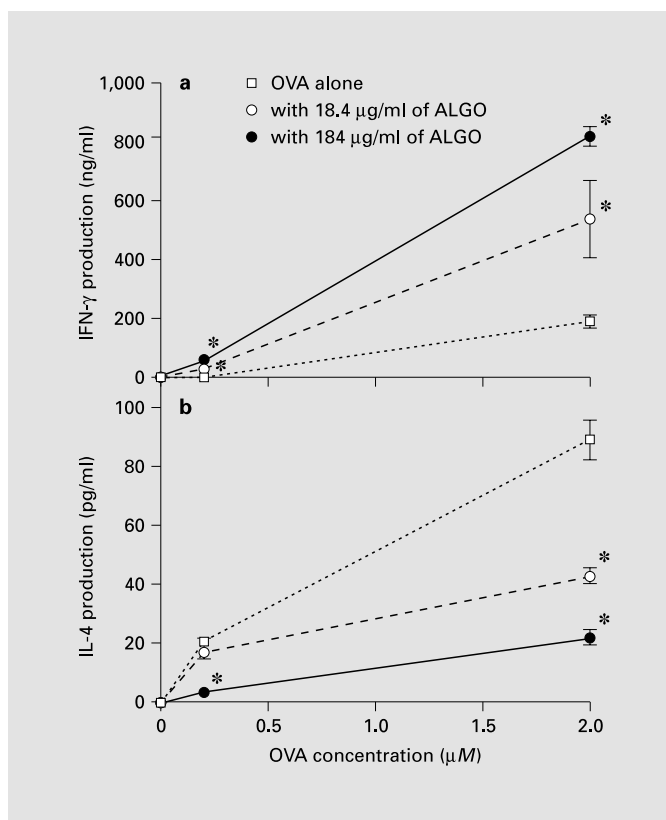


Fig. 6. Effect of ALGO on the Th1 and Th2 development of splenocytes obtained from TCR transgenic mice. Splenocytes from untreated TCR transgenic mice were cultured according to the method described for the experiments in figure 1. IFN- γ (a) and IL-4 (b) production in each supernatant was tested. The result is representative of two independent experiments. * Significant difference compared with the control.

alone was also lower in C3H/HeJ mice than that in C3H/HeN mice. Our animal facilities are maintained for conventional conditions and the mice were always exposed to the environmental microorganisms. The response to an immunized antigen in C3H/HeN mice may be higher than that of C3H/HeJ mice due to the spontaneous adjuvant effect of these microorganisms. Together with these, this result demonstrates that ALGO interacted with lymph node cells via a mechanism other than TLR4.

ALGO Suppressed Th2 Development of Naïve Splenocytes

Our experimental system using BALB/c mice could not examine the effect of ALGO on Th2 development, because their lymph node cells did not produce sufficient

IL-4 for the examination. TCR transgenic mice were therefore used for a similar experiment. Almost all T cells of these mice are specific for OVA, and the splenocytes of these mice can respond intensively to the antigen. As shown in figure 6, T cells cultured with ALGO produced more IFN- γ in response to OVA, but less IL-4.

ALGO Inhibited Antigen-Induced IgE Production in vivo

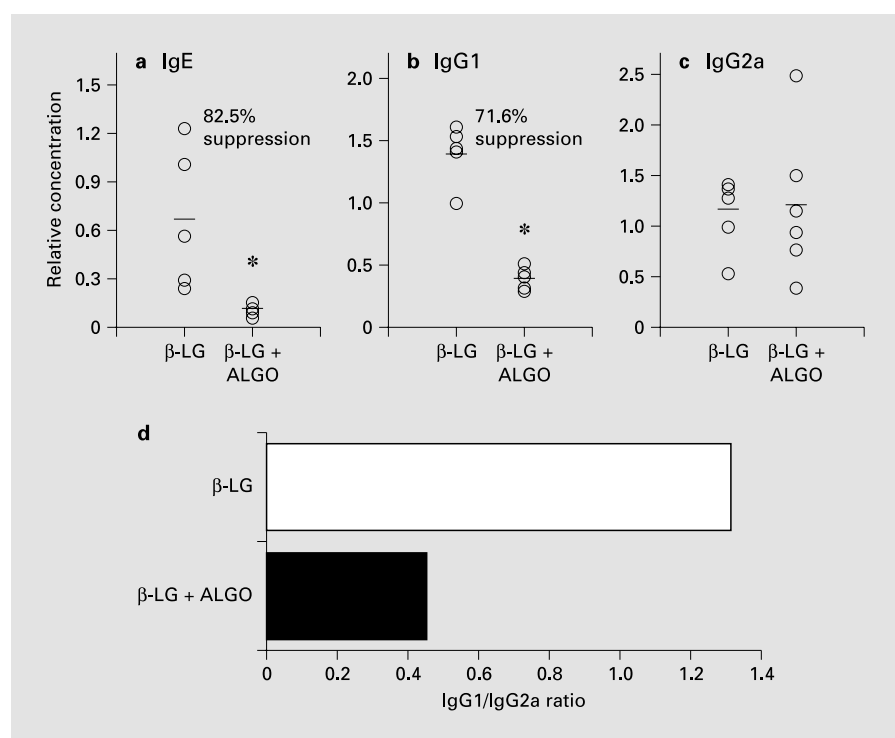
The activity of ALGO for enhancing Th1 and inhibiting Th2 responses was tested in vivo. ALGO significantly downregulated the production of the antigen-specific IgE and IgG1 antibodies (fig. 7a, b). On the other hand, the production of the antigen-specific IgG2a antibody in mice immunized with β -LG and ALGO was at the same level as that of the mice immunized with β -LG alone (fig. 7c). The ratio of IgG1/IgG2a was reduced by the administration of ALGO (fig. 7d), indicating that ALGO could also regulate the Th1 and Th2 balance toward Th1 in vivo.

Discussion

The aim of this study was to find a method to control allergic disorders by regulating the Th1 and Th2 balance. We have examined the Th1-inducing activity of ALGO. Our results demonstrate that ALGO enhanced Th1 development and suppressed Th2 both in vitro and in vivo. These findings suggest the potential use of ALGO as a novel anti-allergic agent.

Alginic acid is a natural polysaccharide present in seaweed and algae; it is widely used as a food material. ALGO is the lyase-lysate of alginic acid, and many physiological functions of alginic acid and ALGO have been demonstrated [9–14]. The results of our previous studies suggested that ALGO can regulate several immune responses [unpublished observation]. In this report, our results demonstrate that ALGO enhanced Th1 differentiation through the upregulation of IL-12 production in murine lymphocyte cultures. The effect of ALGO was found to be dose dependent and was completely abrogated by blockade with IL-12. Although these results strongly indicate the contribution of IL-12 to the activity of ALGO, the participation of other molecules, such as IL-12 p40 homodimer [25] or IL-23 [26], because the antibody used in this experiment was specific for the p40 subunit. Any false activity of ALGO caused by contamination with LPS was inhibited by treating ALGO in a column to remove LPS. In addition, the experiment using

Fig. 7. Effect of ALGO on β -LG-induced antibody production in vivo. BALB/c mice were immunized 3 times with β -LG or β -LG plus ALGO. The anti-sera of these mice were collected 4 weeks after the first immunization. The β -LG-specific antibody titers in the sera were determined by ELISA. The relative antibody concentration was calculated in comparison with the standard serum. The ratio of IgG1/IgG2a was calculated as the geometric mean of the ratio in individual mice. Five or six mice were used in an individual experiment for each group, and similar results were obtained from two independent experiments.



C3H/HeJ also strongly excluded any false activity due to LPS. Furthermore, ALGO inhibited Th2 development in a splenocyte culture of TCR transgenic mice. These findings clearly demonstrate the ability of ALGO to regulate the Th1 and Th2 balance toward Th1 dominance.

Such Th2-type cytokines as IL-4 and IL-5 are known as mediators of the type I allergic disorder. IL-4 induces IgE production [27] and activates mast cells [28], while IL-5 activates eosinophils [28]. Several previous studies have therefore tried to control allergic diseases by enhancing Th1 response, which results in the suppression of Th2 cytokines [4, 5, 7]. It has been shown that IL-12 inhibited IgE production in both IL-4-stimulated human peripheral blood mononuclear cells [29] and mice treated with the anti-IgD antibody [30]. Furthermore, the lactic acid bacterium, *Lactobacillus casei*, which is an IL-12 potentiator, was effective in inhibiting antigen-induced IgE production in vitro and in vivo [4, 5]. Our findings indicate that ALGO also suppressed IgE production and may be an anti-allergic agent.

We further examined the activity of ALGO to enhance IFN- γ production by fractionating the compound on the basis of molecular weight. The results indicate that highly polymerized ALGO had stronger activity than ALGO of lower molecular weight. Since alginic acid is a hardly

digestible polysaccharide for humans [9, 31], it is considered that artificial digestion by an appropriate enzyme would be useful for the efficient uptake of alginic acid from the intestinal lumen. At the present time, we have some preliminary observations indicating that the oral administration of unfractionated ALGO suppressed IgE production in antigen-immunized mice [unpublished observation]. Together with these observations, although ALGO is expected to be an edible anti-allergic agent without any risk, because alginic acid is widely used in many foods, further examination is required to study the appropriate degree of polymerization of ALGO for its efficient absorption and strong activity.

Alginic acid consists of the two types of saccharide, *L*-guluronic acid and *D*-mannuronic acid. Kawada et al. [14] have reported that ALGO enhanced the growth of human keratinocytes and that the activity was dependent not only on the molecular weight of ALGO but also on the sequence of saccharides. Their evidence suggests that the sequence of saccharides in ALGO may also be involved in the activity for inducing Th1 development. Although we have not analyzed the saccharide sequence of ALGO having strong activity, the ratio of *L*-guluronic acid to *D*-mannuronic acid was determined as 47:53.

Macrophages, DC or B cells are possible producers of IL-12. It is considered that these cells may receive a signal from ALGO to enhance IL-12 production. The experiment using fractionated lymph node cells shows that ALGO did not directly enhance IFN- γ production by T cells. On the other hand, the non-B cell fraction, but not the B cell fraction, strongly enhanced IFN- γ production, indicating that macrophages and/or DC might be the target cells of ALGO. These innate immune cells have many receptors for recognizing natural microorganisms. LPS of gram-negative bacteria is known as a strong Th1-inducing agent [32]. Recent studies have reported that murine cells recognized LPS via TLR4 [16]. Our result obtained from the experiment with C3H/HeJ mice, which cannot respond to LPS due to their lack of TLR4 function, clearly shows that ALGO interacted with the lymph node cells via a mechanism other than TLR4. TLR is one of the PRR and is known to recognize some bacterial membrane components or DNA [16–18, 33]. It is known that macrophages and DC express several PRR, including TLR4. Although no receptor which recognizes uronic acid, such as alginic acid, has been reported, it is considered that ALGO may also be recognized by some TLR or other PRR such as the lectin receptor which is expressed on the surface of macrophages and/or DC. Tsuji et al. [6] have demonstrated that λ -carrageenan was recognized by either a TLR4-dependent or -independent mechanism, and regulated IgE production. Furthermore, Shibata et al. [15] have reported that the phagocytosis of chitin particles induced the upregulation of IL-12 by murine splenocytes. It is considered that phagocytosis was also involved in the upregulation of IFN- γ production by ALGO, because high-molecular-weight ALGO had stronger activity. It has been considered that the signals via TLR4 are necessary to induce endotoxin shock. Our results show that ALGO had an effect without TLR4, suggesting that ALGO would not induce any endotoxin-like shock. It is expected that ALGO would be a harmless agent for humans.

The activity of ALGO in vivo was also investigated. Our results demonstrate that ALGO was also able to inhibit IgE production, and control the balance of Th1 and Th2 toward Th1 dominance in vivo. It had been considered that ALGO would enhance IgG2a production and suppress IgG1 in vivo, because ALGO upregulated IFN- γ production in vitro. However, as shown in figure 7, ALGO downregulated IgE and IgG1 production without enhancing IgG2a production. We consider that the enhancing effect of ALGO on IgG2a production was negated by the suppression of Th2 cytokines, which resulted in downregulation of all classes of antibody production.

This observation demonstrates that the administration of ALGO can inhibit IgE production without causing hyper-Th1-type antibody production. In this experiment, ALGO and antigen were co-immunized, so it cannot be excluded that ALGO directly affected the immunogenicity of antigen. We however considered that ALGO modulated the activity of DC or macrophages, because ALGO upregulated IL-12 production independent of the antigen (table 1). In addition, ALGO also affected the IgE production when ALGO and antigen were administered by separate route (p.o. or i.p., respectively) [unpublished observation]. On the other hand, ALGO cannot inhibit the already established immune response such as IgE production by this mechanism. Nevertheless, ALGO should be useful for the treatment of allergic diseases, because allergy is induced and becomes severe following repeated exposure to allergens. In this process, once Th2 response has been established, it increases the intensity by itself more and more, leading to IgE hyperproduction. ALGO can modulate this cycle to Th1 dominance. Furthermore, ALGO can be used for prophylaxis, because it is considered that ALGO is harmless for humans, even if taken daily, because it does not induce TLR4 signaling. In addition, the Th2 cytokines, IL-4 and IL-5, are known to be critical factors for activating mast cells and eosinophils [28]. Together with these, ALGO may be beneficial for regulating allergic disorders by suppressing not only IgE production but also the activation of mast cells and eosinophils.

In conclusion, the results of the present study clearly demonstrate that ALGO, an edible oligosaccharide, can suppress Th2 response and inhibit antigen-induced IgE production. This result suggests a possible use of ALGO in preventing IgE-mediated allergic disorders. A new potential use of ALGO is therefore as an edible anti-allergic agent. It is also considered that ALGO could act as an anti-tumor, anti-bacterial and anti-viral agent, because several previous studies have shown that Th1-potentiating agents had such activities, probably due to their ability to induce IL-12 production.

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