



Fucoidan prevents C ϵ germline transcription and NF κ B p52 translocation for IgE production in B cells

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Abstract

Fucoidan, a dietary fiber contained in seaweed, reduces the increase of antigen-specific IgE in mice exposed to ovalbumin. In this study, we investigated the effect of fucoidan on IgE production and intracellular events in B cells *in vitro*. Fucoidan inhibited the production of IgE and C ϵ germline transcription in murine B cells induced by IL-4 (100 ng/ml) and anti-CD40 antibodies (10 μ g/ml), whereas it stimulated cell proliferation. A significant effect of fucoidan on IgE production was observed when B cells were stimulated with a higher dose (5 μ g/ml) of anti-CD40 antibodies, but not when stimulated with lower doses (1.25, 2.5 μ g/ml), regardless of the IL-4 concentrations. Moreover, nuclear translocation of NF κ B p52, but neither that of NF κ B p65, nor the phosphorylation of JAK1 and STAT6 was reduced by fucoidan. These results suggest that fucoidan inhibited IgE production by preventing the NF κ B p52-mediated pathways activated by CD40.

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A high serum IgE concentration is one of the critical characteristics of atopic diseases, such as atopic dermatitis, asthma, and allergic rhinitis [1–3]. Clinical observations have suggested a correlation between serum IgE levels and the extent and/or the severity of a disease, especially with cutaneous lesions [4,5]. The antigen-specific IgE bound to Fc ϵ receptors expressed on mast cells induces the release of inflammatory mediators, such as histamine, leukotrienes, and prostaglandins in response to the binding of multivalent antigens, which subsequently cross-links the Fc ϵ receptors. These factors cause the immediate hypersensitivity reaction, and the late-phase inflammatory reaction involving T cells and eosinophils [6]. Recent studies have shown that the ligation of IgE itself on Fc ϵ receptors without antigen can stimulate the release of such inflammatory

mediators from mast cells [7,8]. Hence, the inhibition of IgE production is an important therapeutic target for atopic diseases.

IgE synthesis in B cells requires two signals; one triggered by interleukin-4 (IL-4), and the other by the ligation of cell surface CD40 by activated T cells. The binding of IL-4 to the IL-4 receptor triggers the activation of Janus kinase (JAK)1 and JAK3 tyrosine kinases, which then phosphorylate the tyrosine residues of signal transducer and activator of transcription 6 (STAT6). The phosphorylated STAT6 homodimerizes and subsequently translocates into the nucleus and promotes transcriptions of target genes. The crosslinking of CD40 by CD40 ligands expressed on T cells induces the migration of nuclear factor- κ B (NF κ B) to the nucleus. The NF κ B family is composed of five members: p65, RelB, c-Rel, p50, and p52. They form various dimeric complexes and activate genes specifically targeted in the nucleus [9]. The signals induced

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by the IL-4 receptor and those by CD40 cooperatively promote C ϵ germline transcription (ϵ GT) [10,11]. C ϵ germline transcripts associate with DNA in the genomic switch region of the IgE locus to form DNA–RNA hybrid structures, which are subject to the deletional switch recombination by excision-repair nucleases [12,13]. Thus, ϵ GT initiates IgE class switch recombination.

Fucoidan, an ingredient of seaweed, is composed of a polymer of α 1 $\rightarrow$ 3-linked L-fucose, with sulfate groups on some of the fucose residues at the 4 positions [14]. It shows a variety of biological effects in mammals, including the prevention of leukocyte rolling by binding to L-selectin [15], and the inhibition of low-density lipoprotein uptake by macrophage through the prevention of their binding to the scavenger receptor [16]. Recently, Maruyama *et al.* have reported that fucoidan reduced the concentrations of both IL-4 and IL-13 in bronchoalveolar lavage fluid, and inhibited the increase of antigen-specific IgE in ovalbumin-induced mouse airway hypersensitivity [17]. On the other hand, Eiwegger *et al.* have reported that fucoidan induced IL-4 production in CD3⁺CD4⁺ cord blood mononuclear cells *in vitro* [18]. Thus, the impairment of IL-4 production or Th1/Th2 balance may not explain the whole mechanism of how fucoidan affects IgE production.

In this study, we clarified the mechanism of fucoidan to inhibit IgE production by evaluating the level of ϵ GT, intracellular signal transductions induced by IL-4 and anti-CD40 antibodies in murine B cells cultured in the presence of fucoidan.

Materials and methods

Animals. Male BALB/c mice were purchased from Charles River Japan (Yokohama, Japan). They were maintained in a specific pathogen-free environment in closed racks with free access to food and water in the Institute of Laboratory Animal Science, Hiroshima University. This study was carried out in accordance with the Guideline for Animal Experiments in Hiroshima University and the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University.

Preparation and culture of B cells. The method of B cell culture was described previously [19]. Briefly, spleens were isolated from 8 to 10-week-old mice and minced in Iscove's modified Dulbecco's medium (IMDM, Invitrogen, Carlsbad, CA) with 10% heat-inactivated fetal bovine serum (Invitrogen), penicillin G (10 IU/ml, Invitrogen), and streptomycin (100 μ g/ml, Invitrogen). Lymphocyte suspensions were obtained with a nylon mesh filter (Becton Dickinson Labware, Franklin Lakes, NJ) and a density separation medium (Lympholyte-M; Cedarlane Laboratories, Hornby, Canada). T cells, monocytes/macrophages, and NK cells were then depleted from the splenocyte suspensions by incubating them with anti-CD43 (ly-48) magnetic microbeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) for 20 min at 4 °C, washing with PBS, and passing through the magnetic-activated cell sorting (MACS) with autoMACS column (Miltenyi Biotec) according to the manufacturer's instructions. The isolated B cells, of which more than 97% were CD19-positive cells, were distributed into a 96-well tissue culture plate (Becton–Dickinson) at 4×10^5 cells/0.2 ml per well and maintained for indicated periods at 37 °C in an atmosphere containing 5% CO₂ in the presence or absence of IL-4 (R&D Systems, Minneapolis, MN), anti-CD40 antibodies (Serotec, Oxford, UK), and fucoidan derived from *Cladosiphon novae-caledonias*

Kylin (Tanglewood, Hiroshima, Japan) or *Fucus vesiculosus* (Sigma, St. Louis, MO) in the medium described above.

Measurement of IgE. B cells were cultured for 4 or 7 days as indicated. The supernatant of culture medium was collected by centrifugation and stored at –20 °C until use. The concentrations of IgE in the culture supernatant were determined by means of a mouse IgE ELISA kit purchased from Bethyl Laboratories (Montgomery, TX), following the manufacturer's instructions.

The assay for cell proliferation. To evaluate the proliferation of B cells, the amount of bromodeoxyuridine (BrdU) uptake at 3 days culture of B cells was measured by means of a cell proliferation ELISA BrdU Kit (Roche Diagnostics GmbH, Penzberg, Germany), following the manufacturer's instructions. The amounts of proliferating cells were expressed as ratios to that in the culture without fucoidan.

Measurement of ϵ GT levels by real-time RT-PCR. The levels of ϵ GT in B cells cultured with IL-4 (100 ng/ml) and anti-CD40 antibodies (10 μ g/ml) in the presence or absence of fucoidan (100 μ g/ml) for 1 or 3 days were evaluated by quantitative real-time RT-PCR with an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster City, CA) as described previously [20]. Total RNA was isolated with a RNeasy Mini kit (QIAGEN K.K., Tokyo, Japan) and subjected to the first-strand cDNA synthesis by a Superscript II first-strand synthesis system for RT-PCR (Invitrogen). The design of primers and TaqMan probes for C ϵ germline transcript were based on a previous report [21], and synthesized by Operon Biotechnologies (Tokyo, Japan). Measurement of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels, as an internal standard for calibration, was performed with TaqMan rodent GAPDH control reagent (Applied Biosystems). The expressions of each mRNA were quantified by relating the PCR threshold cycle obtained from tissue samples to amplicon-specific standard curves. Amplification was performed for 1 cycle of a sequential incubation at 50 °C for 2 min and 95 °C for 10 min, followed by subsequent 60 cycles of a consecutive incubations at 95 °C for 15 s and 60 °C for 1 min.

Western blotting. B cells were cultured for indicated periods with IL-4 and anti-CD40 antibodies in the presence or absence of fucoidan and lysed in cell lysis buffer (Cell Signaling Technology, Danvers, MA) with 1 mM phenylmethylsulfonyl fluoride (Wako Pure Chemical, Osaka, Japan) and used as whole cell lysate. Nuclear extracts were prepared with the Nuclear Extract Kit (Active Motif, Carlsbad, CA) according to the manufacturer's instructions. The samples (10 μ g/lane) were mixed with an equal volume of 2 $\times$ sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) buffer containing 10% 2-mercaptoethanol (v/v, Sigma), boiled for 5 min, subjected to SDS–PAGE, and electrotransferred onto nitrocellulose membranes (Pall, East Hills, NY). Following preincubation with blocking reagent (5% BSA (w/v) in Tris-buffered saline with Tween 20 (TBST)) for 3 h at room temperature, membranes were preincubated with primary antibodies (1:1000 dilution) for overnight at 4 °C. Rabbit anti-phospho-STAT6 (Tyr 641), rabbit anti-phospho-JAK1 (Tyr 1022/1023), rabbit anti-JAK1, rabbit anti-NF κ B p65, rabbit anti-NF κ B p100/p52, and rabbit anti- β -actin antibodies were purchased from Cell Signaling Technology (Danvers, MA). Rabbit anti-STAT6 antibody and rabbit anti-Oct-1 antibody were purchased from EMD Biosciences (San Diego, CA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Membranes were washed by TBST, incubated with a horseradish peroxidase-conjugated anti-mouse, or anti-rabbit secondary antibody (1:2000 dilution, Cell Signaling Technology) for 1 h, and developed using a chemiluminescence reagent (ECL plus) (GE Healthcare Bio-Sciences, Piscataway, NJ).

Statistics. Data were presented as means $\pm$ SEM. The significance of differences among the fucoidan treatment, and those among the fucoidan treatment in different time points or in different doses of IL-4 or anti-CD40 antibodies was determined by one-way ANOVA, and two-way ANOVA, respectively. *Post hoc* analyses were performed by the Bonferroni's post-test. The significance of differences between two groups was evaluated using Student's *t*-test. A value of $p < 0.05$ was considered as significant.

Results

Fucoidan prevents IgE production induced by IL-4 and anti-CD40 antibodies in B cells

To investigate the role of fucoidan in the modulation of IgE production *in vitro*, B cells were cultured with IL-4 and anti-CD40 antibodies in the presence or absence of various concentrations of fucoidan derived from *Cladosiphon novae-caledonias* Kylin (0, 1, 10, and 100 $\mu\text{g/ml}$) or *Fucus vesiculosus* (0 and 15 $\mu\text{g/ml}$) for 4 or 7 days. Fucoidan derived from *Cladosiphon novae-caledonias* Kylin significantly reduced IgE production in a dose-dependent manner in the culture for 4 days, and more clearly in the culture for 7 days (Fig. 1a). Fucoidan derived from *Fucus vesiculosus* also showed almost the same effect on B cells when cultured for 7 days (Fig. 1b). We, therefore, employed fucoidan derived from *Cladosiphon novae-caledonias* Kylin for the following experiments.

Fucoidan stimulates the proliferation of B cells in the presence of IL-4 and anti-CD40 antibodies

To determine the effect of fucoidan on cell proliferation, B cells were cultured with IL-4 and anti-CD40 antibodies in the presence or absence of fucoidan (10 and 100 $\mu\text{g/ml}$) for 3 days. Fucoidan increased the amount of BrdU-labeled cells in a dose-dependent manner (Fig. 2), indicating that the impairment of IgE production by fucoidan is not due to the reduction of cell numbers or cytotoxicity for B cells.

Fucoidan prevents ϵGT induced by IL-4 and anti-CD40 antibodies in B cells

To study the effect of fucoidan on IgE class switching, we measured the levels of ϵGT in B cells cultured with

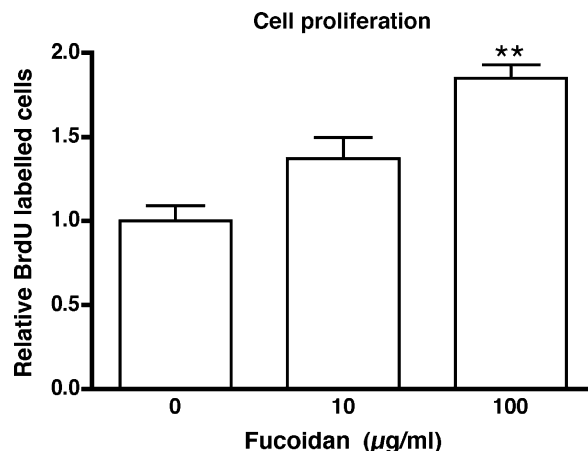


Fig. 2. Fucoidan enhanced the proliferation of B cells cultured with IL-4 and anti-CD40 antibodies. B cells were cultured with IL-4 (100 ng/ml) and anti-CD40 antibodies (10 $\mu\text{g/ml}$) in the presence of 0, 10, and 100 $\mu\text{g/ml}$ fucoidan for 3 days. BrdU was added to the culture 3 h before fixation. The amount of BrdU incorporated into cells was determined by ELISA. Fucoidan enhanced the amount of cellular BrdU in a dose-dependent manner. Data are means $\pm$ SEM and representative results from 3 separate experiments. ** $P < 0.01$ vs Fucoidan 0 $\mu\text{g/ml}$.

IL-4, anti-CD40 antibodies, and various concentrations of fucoidan for 2 days. The induction of ϵGT was suppressed by the treatment of fucoidan in a dose-dependent manner (Fig. 3). These results suggested that the inhibitory effect of fucoidan on IgE production was due to the prevention of IgE class switching in B cells.

Time course of the inhibitory effect of fucoidan on IgE production

To determine the critical time of fucoidan to inhibit IgE production, fucoidan (100 $\mu\text{g/ml}$) was added at different time points in the culture of B cells for 7 days. As shown in

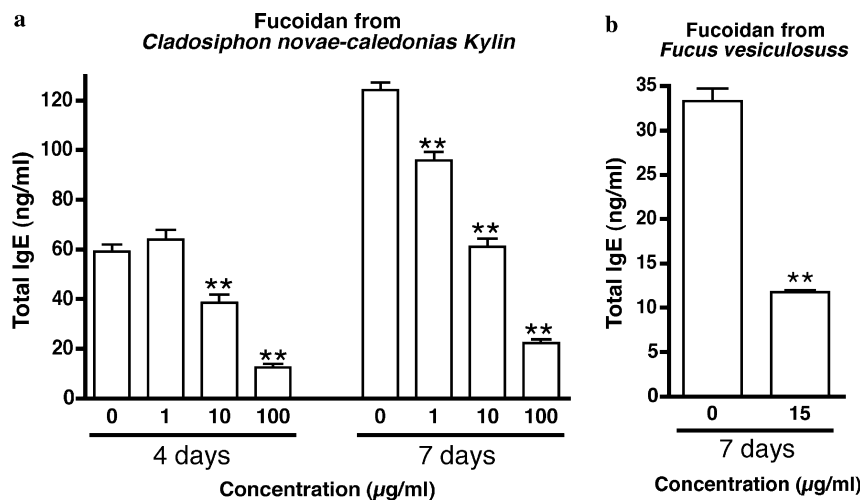


Fig. 1. Fucoidan prevents the increase of IgE production by B cells stimulated with IL-4 and anti-CD40 antibodies. B cells isolated from spleens were cultured with IL-4 (100 ng/ml) and anti-CD40 antibodies (10 $\mu\text{g/ml}$) in the presence of 0, 1, 10, 15, and 100 $\mu\text{g/ml}$ fucoidan for 4 or 7 days. IgE concentrations in culture supernatants were determined by ELISA. Fucoidan derived from *Cladosiphon novae-caledonias* Kylin significantly inhibited IgE production in a dose dependent manner. Fucoidan from *Fucus vesiculosus* also prevented the production of IgE as well. Data are means $\pm$ SEM and representative results from five separate experiments. ** $P < 0.01$ vs control group.

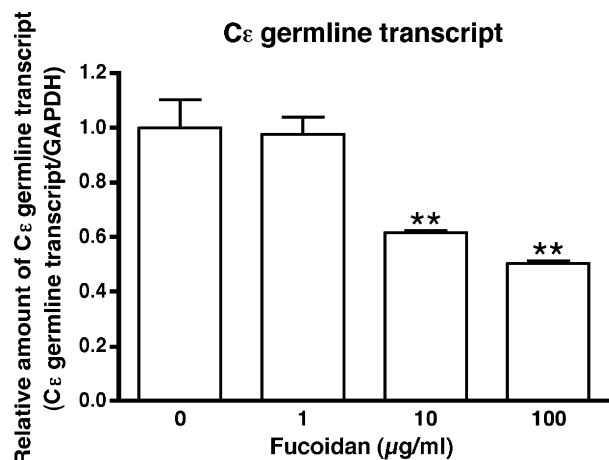


Fig. 3. Fucoidan suppressed the induction of ϵ GT in B cells stimulated by IL-4 and anti-CD40 antibodies. B cells were cultured with IL-4 (100 ng/ml) and anti-CD40 antibodies (10 μ g/ml) in the presence of 0, 1, 10, and 100 μ g/ml fucoidan for 2 days. The transcriptional levels of C ϵ germline were determined by real-time RT-PCR, normalized by GAPDH, and expressed as ratios to that in the absence of fucoidan (relative value, 1.0). Fucoidan significantly inhibited ϵ GT in a dose-dependent manner. Data are means $\pm$ SEM and representative results from three separate experiments. ** P < 0.01 vs Fucoidan 0 μ g/ml.

Fig. 4a, the maximum effect was observed when fucoidan was added at time points leading up to 24 h after the start of the culture. A modest, but significant inhibition was achieved when fucoidan was added at 48 h. However, the addition of fucoidan to B cells, that were pretreated with IL-4 and anti-CD40 antibodies for 72 h in the absence of fucoidan, showed no apparent effect on the increase of IgE. In accordance with IgE production, the levels of ϵ GT in B cells, cultured with fucoidan for 24 h within 48 h from the beginning of stimulation, were significantly lower than those cultured without fucoidan. However, no effect was observed when fucoidan was added after the period of culture with IL-4 and anti-CD40 antibodies for 72 h (Fig. 4b).

Fucoidan inhibited NF κ B p52-mediated pathway in B cells

B cells produced IgE, when both IL-4 receptor and CD40 were stimulated in a dose-dependent manner. To investigate the action mechanism of fucoidan for the inhibition of IgE production, we treated B cells with various doses of IL-4 (50, 100, and 200 ng/ml) and anti-CD40 antibodies (1.25, 2.5, and 5 μ g/ml) in the presence or absence of fucoidan (100 μ g/ml). When B cells were treated with a high dose (5 μ g/ml) of anti-CD40 antibodies, the production of IgE with any dose of IL-4 was significantly inhibited by fucoidan (Fig. 5a). However, fucoidan did not significantly prevent IgE production, when B cells were stimulated with low doses (1.25, 2.5 μ g/ml) of anti-CD40 antibodies and a high dose (100 ng/ml) of IL-4 (Fig. 5b). These results suggested that the effect of fucoidan is largely dependent on the pathway induced by CD40.

To further clarify the mechanism of the effect of fucoidan on intracellular events mediated by IL-4 receptors

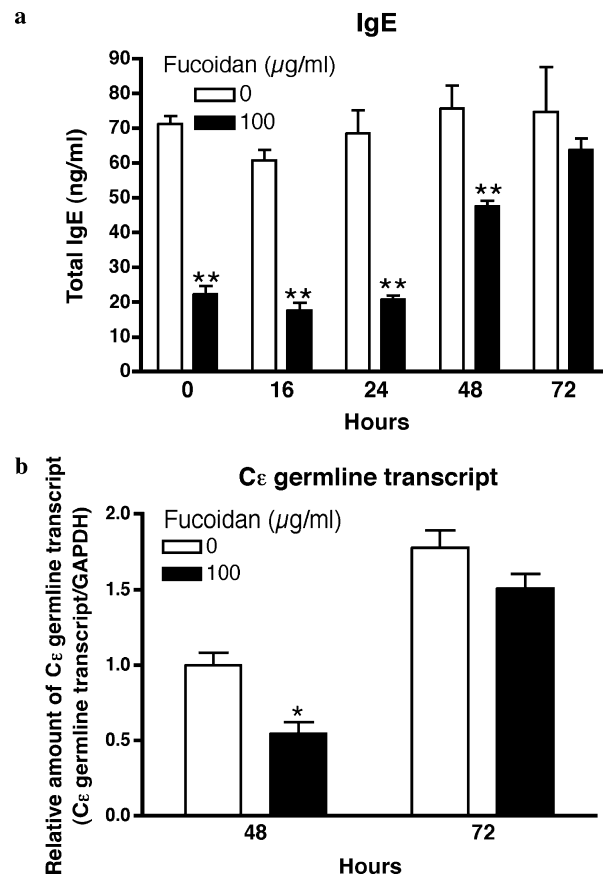


Fig. 4. Time course of the effect of fucoidan on the reaction of B cells to IL-4 and anti-CD40 antibodies. B cells were stimulated with IL-4 (100 ng/ml) and anti-CD40 antibodies (10 μ g/ml) followed by the addition of fucoidan (100 μ g/ml) at various time points. The amounts of IgE in culture supernatants were measured at 7 days after the start of culture. Relative amounts of C ϵ germline transcript were determined after B cells were cultured with fucoidan for 24 h, following the pretreatment with IL-4 and anti-CD40 antibodies for 48 or 72 h. The production of IgE was significantly inhibited when fucoidan was added by 48 h from the start of stimulation with IL-4 and anti-CD40 antibodies (a). The levels of ϵ GT in culture, where fucoidan was added at 48 h after the start of stimulation, were significantly lower than those in the culture without fucoidan. However, no effect of fucoidan was observed when added at 72 h (b). Data are means $\pm$ SEM and representative results from three separate experiments. * P < 0.05, ** P < 0.01 vs Fucoidan 0 μ g/ml.

and CD40 in B cells, we investigated the effect of fucoidan on the phosphorylation of JAK1 and STAT6, and the nuclear translocation of NF κ B protein. Western blotting for JAK1 and STAT6 revealed that they were both phosphorylated in response to exogenous IL-4 and anti-CD40 antibodies at no later than 15 min and the degree of phosphorylation remained the same or slightly elevated towards 60 min. No apparent effect of fucoidan was observed regardless of the culture periods (Fig. 6) or the concentration of IL-4 (data not shown), suggesting that fucoidan did not affect the pathway induced by IL-4. Studies of nuclear proteins by Western blotting showed that both NF κ B p65 and p52 were translocated to the nucleus in response to exogenous IL-4 and anti-CD40 antibodies at 1 h. The amount of NF κ B p65 in the nucleus then

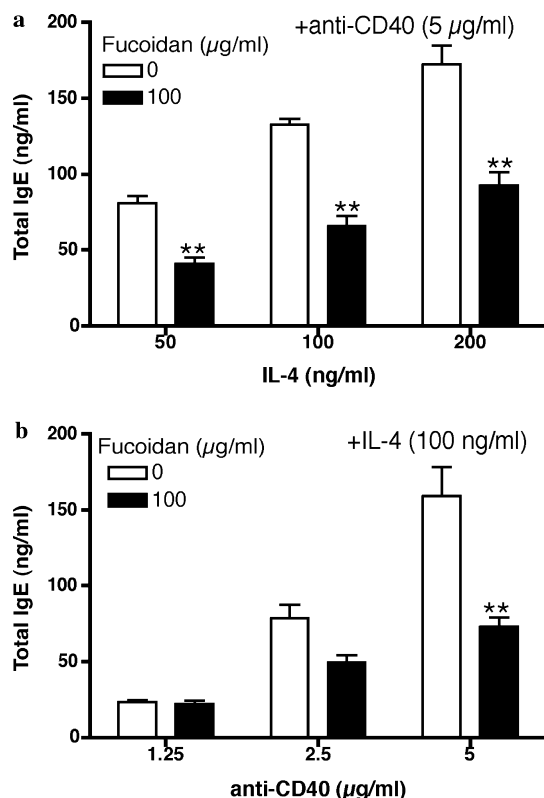


Fig. 5. The effect of fucoidan on IgE production induced by various dose-combinations of anti-CD40 antibodies and IL-4. B cells were stimulated with either IL-4 (50, 100, and 200 ng/ml) and anti-CD40 antibodies (5 µg/ml, a), or IL-4 (100 ng/ml) and anti-CD40 antibodies (5, 2.5, and 1.25 µg/ml, b) in the presence (100 µg/ml) or absence of fucoidan for 7 days. Fucoidan significantly reduced IgE production in response to a high dose of anti-CD40 antibodies (5 µg/ml) and various doses of IL-4, but not those in response to low doses (1.25, 2.5 µg/ml) of anti-CD40 antibodies and a high dose of IL-4 (100 ng/ml). Data are means $\pm$ SEM and representative results from three separate experiments. ** $P < 0.01$ vs respective control.

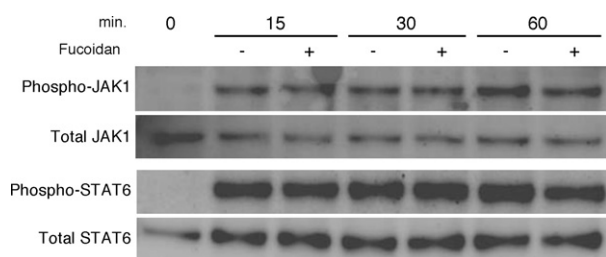


Fig. 6. Fucoidan did not alter the phosphorylation of STAT6 and JAK1 in B cells induced by IL-4 and anti-CD40 antibodies. B cells were stimulated with IL-4 (100 ng/ml) and anti-CD40 antibodies (10 µg/ml) in the presence (+) or absence (–) of fucoidan (100 µg/ml) for 15, 30 or 60 min. The levels of phosphorylated STAT6 and JAK1 protein were determined by Western blotting. Total amounts of STAT6 and JAK1 were included as loading controls. The phosphorylation of STAT6 (a) and JAK1 (b) was not altered by fucoidan. This is a representative result from three separate experiments conducted. Similar results were obtained when the concentration of IL-4 was 10 ng/ml (data not shown).

decreased towards 46 h and was not affected in the presence of fucoidan (100 µg/ml). On the other hand, the amount of nuclear translocation of NFκB p52 increased towards 46 h.

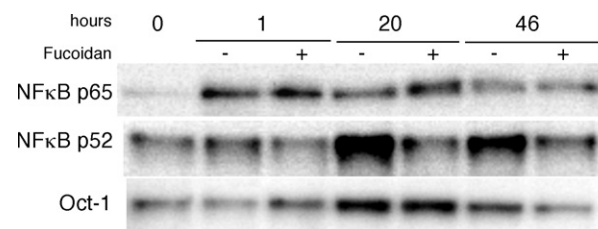


Fig. 7. Fucoidan prevented the nuclear translocation of NFκB p52, but not that of NFκB p65 in B cells induced by IL-4 (100 ng/ml) and anti-CD40 antibodies (10 µg/ml). B cells were stimulated with IL-4 and anti-CD40 antibodies in the presence (+) or absence (–) of fucoidan (100 µg/ml) for 1, 20 or 46 h. The loaded samples were prepared from the nucleus. The levels of nuclear translocation of NFκB p65 and NFκB p52 were determined by Western blotting. An Oct-1 immunoblotting is included as a loading control. The nuclear translocation of NFκB p52 was increased towards 46 h. Fucoidan strongly prevented the nuclear translocation of NFκB p52 at 20 and 46 h, but did not affect that of NFκB p65. This is a representative result from four separate experiments conducted.

Moreover, the increase was inhibited by fucoidan at 20 and 46 h (Fig. 7).

Discussion

In the present study, we demonstrated that fucoidan, a sulfate polysaccharide contained in seaweed, inhibited IgE production, εGT, and the nuclear translocation of NFκB p52 in B cells induced by IL-4 and anti-CD40 antibodies *in vitro*. Fucoidan is a polymer of L-fucose with sulfate groups and its structure varies from species to species. Previous studies have reported that the biological effect of fucoidan depends on the origin of the seaweed [22,23]. However, we showed that both fucoidan derived from *Cladosiphon novae-caledonias* Kylin and that from *Fucus vesiculosus* prevented the IgE production in B cells. Moreover, Maruyama *et al.* have reported that fucoidan from *Undaria pinnatifida* Sporophylls also suppressed IgE levels in ovalbumin-sensitized mice [17]. Taken together, the inhibitory action on IgE production is likely to be a common property of fucoidan.

IgE production was inhibited when fucoidan was added at time points leading up to 48 h. However, it was no longer inhibited when fucoidan was added at 72 h from the beginning of the culture with IL-4 and anti-CD40 antibodies. In accordance with IgE production, the treatment of B cells with fucoidan prevented the expression of εGT in B cells prestimulated with IL-4 and anti-CD40 antibodies for 48 h, but not those prestimulated for 72 h. These results suggest that fucoidan inhibits the IgE production through the impairment of class switch recombination to IgE. Moreover, previous studies have shown that the expression of IgE on the surface of murine B cells was detected after 72 h of the culture with IL-4 and anti-CD40 antibodies [24,25]. We also detected the induction of mature Cε transcription in B cells stimulated with IL-4 and anti-CD40 antibodies from 54 h by real-time RT-PCR (data not shown). Thus, once B cells are engaged to produce IgE

by completing immunoglobulin class switching, fucoidan may no longer prevent IgE production.

When B cells were stimulated with a high dose (5 µg/ml) of anti-CD40 antibodies, fucoidan strongly inhibited IgE production in the presence of various doses (50, 100, and 200 ng/ml) of IL-4. However, no or only weak inhibition was induced when B cells were activated by low doses (1.25, 2.5 µg/ml) of anti-CD40 antibodies in the presence of 100 ng/ml IL-4. Moreover, the phosphorylation of JAK1 and STAT6 induced by the activation IL-4 receptor was not affected by fucoidan. On the other hand, the nuclear translocation of NFκB p52, which is induced by the activation of CD40, was inhibited by the treatment with fucoidan. These results indicate that fucoidan inhibits the signal induced by the activation of CD40, rather than those by IL-4 or those activated constitutively.

The activation of CD40 induces gene transcriptions via two pathways; one mediated by NFκB p65/p50 complex and the other by NFκB p52/relB complex. In the cytoplasm, NFκB p65/p50 is present with inhibitory NF-κB (IκB) as a quiescent form in the absence of stimulation. The CD40 activation induces the phosphorylation of IκB disclosing a nuclear localization sequence of NFκB p65/p50 and its migration from the cytoplasm to the nucleus. Another pathway of NFκB activation, involving NFκB p52 production, has been recently identified. It is specifically mediated by a subset of tumor necrosis factor (TNF) receptor family members, including CD40, B cell activating factor belonging to the TNF family (BAFF) receptor, and lymphotoxin receptor [26]. They induce the activation of NFκB-inducing kinase, IκB kinase α, and the conversion of p100 precursor protein to NFκB p52. The p52 dimerizes with RelB and translocates to the nucleus. IgE germline promoter contains both a NFκB p65/p50 binding domain and a NFκB p52/relB binding domain [27]. The NFκB p52 pathway regulates the differentiation of B cells, peripheral lymphoid organogenesis, and immunoglobulin production [9]. NFκB p52 deficient mice show the impairment of antigen-induced production of immunoglobulin, including IgG1 [28]. Of these pathways of NFκB, the nuclear translocation of p52, but not that of p65, was inhibited by fucoidan. Thus, the inhibition of NFκB p52 pathway appears to be the mechanism of fucoidan to inhibit the production of IgE in B cells induced by IL-4 and CD40 activation.

The direct target of fucoidan on the plasma membrane to inhibit IgE production is a matter of discussion. Several proteins, including L-selectin and scavenger receptor class A, have been identified to associate with fucoidan [29,30]. B cells express L-selectin, and ligands for scavenger receptors bind to B cells [31]. Moreover, we have confirmed the expression of mRNA for scavenger receptor class A in B cells by RT-PCR (data not shown). However, the blocking of L-selectin and scavenger receptor class A by respective antibodies did not inhibit the effect of fucoidan (data not shown). The identification of such a receptor may enable us to prevent not only the aggravation, but also the onset of atopic diseases themselves.

We have shown that fucoidan stimulates the proliferation of B cells and prevents the activation of the NFκB p100/p52 pathway in response to IL-4 and anti-CD40 antibodies. However, Caamaño et al. have reported that B cell proliferation by anti-CD40 antibodies was moderately reduced in NFκB p100/p52 deficient mice [32]. This discrepancy may be due to the stimulation of scavenger receptors which has been shown to induce the proliferation of macrophages [33]. Since B cells also express scavenger receptor class A, and fucoidan binds to scavenger receptors, the effect of fucoidan for cell proliferation via scavenger receptor may be larger than its effect on NFκB p100/p52 pathway to inhibit cell proliferation.

In summary, fucoidan suppressed IL-4 and anti-CD40 antibody-induced IgE production, when incubated with B cells before their class switching to IgE, by inhibiting εGT via the NFκB p52 pathway. It was recently reported that the fucoidan concentration in plasma from volunteers who orally received 2.25 g fucoidan per day reached 12.9 µg/ml, which is equivalent to the effective dose in this study [34]. Fucoidan may thus exert a protective effect against diseases caused and/or aggravated by IgE.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bbrc.2006.08.009](https://doi.org/10.1016/j.bbrc.2006.08.009).

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