ORIGINAL ARTICLE

Cellulose Sulfate Suppresses Immunoglobulin E Production by Murine B Lymphocytes In Vitro

S Morioke[1], T Hiragun,[1] Y Yanase,[1] K Uchida,[1] H Suzuki,[1,2] K Iwamoto,[1] M Hide[1]

[1]Department of Dermatology, Division of Molecular Medical Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan
[2]Faculty of Human Science, Hiroshima Bunkyo Women's University, Hiroshima, Japan

Abstract

Background: Immunoglobulin (Ig) E plays an important role in the pathogenesis of allergic diseases such as atopic dermatitis and allergic asthma. We previously reported that a sulfate polysaccharide, fucoidan, suppressed IgE production by murine B cells in vitro. However, the mechanism by which fucoidan suppresses IgE production remains unclear.
Objective: We incorporated sulfate groups into cellulose and studied their biological characteristics in vitro to explore the possibility of converting biologically neutral polysaccharides to active reagents with antiallergic functions.
Material and Methods: Cellulose was chemically processed using N,N-dimethylformamide (DMF) and DMF-sulfurtrioxide and recovered as cellulose sulfate with a molecular weight of around 10 kDa. We then studied the effect of cellulose sulfate on IgE production from B cells, IgE class-switching, and populations of IgE-secreting B cells prepared from murine spleen. We also investigated the effects of sulfated cellulose on the production of interleukin (IL) 4 and interferon (IFN) γ and the expression of T-bet mRNA by splenic T cells. The cytotoxicity of cellulose sulfate was also examined.
Results: Cellulose sulfate suppressed IgE production in B cells stimulated with IL-4 and anti-CD40 antibody by inhibiting IgE class-switch recombination and decreasing the number of IgE-secreting B cells in vitro. Moreover, both cellulose sulfate and fucoidan suppressed IL-4 production and enhanced IFN-γ production by murine T cells stimulated with anti-CD3 and anti-CD28 antibodies, despite the decrease in T-bet mRNA expression.
Conclusions: Cellulose gains an antiallergic effect on B cells and T cells with the addition of sulfate groups.

Key words: Cellulose sulfation. T cells. B cells. IgE. Class-switch recombination.

Resumen

Antecedentes: La inmunoglobulina (Ig) E tiene un papel importante en la patogenia de las enfermedades alérgicas como la dermatitis atópica y el asma alérgica. En un trabajo anterior se notificó que un polisacárido sulfatado, el fucoidán, suprimió la producción de IgE por parte de linfocitos B murinos in vitro. No obstante, el mecanismo mediante el cual fucoidán suprime la producción de IgE sigue siendo incierto.
Objetivo: Se incorporaron grupos sulfato a la celulosa y se analizaron sus características biológicas in vitro para investigar la posibilidad de convertir polisacáridos biológicamente neutros en reactivos activos con funciones antialérgicas.
Materiales y métodos: La celulosa se procesó químicamente mediante N,N-dimetilformamida (DMF) y DMF-trióxido de azufre y se recuperó en forma de sulfato de celulosa con un peso molecular de unos 10.000 Da. A continuación se estudió el efecto del sulfato de celulosa sobre la producción de IgE por parte de los linfocitos B, el cambio de clase de IgE y las poblaciones de linfocitos B secretores de IgE de bazo murino. También se investigaron los efectos del sulfato de celulosa sobre la producción de interleucina (IL) 4 e interferón (IFN) γ y la expresión de ARNm T-bet por parte de linfocitos T esplénicos. Además, se analizó la citotoxicidad del sulfato de celulosa.
Resultados: El sulfato de celulosa suprimió la producción de IgE por parte de linfocitos B estimulados con IL-4 y anticuerpos anti-CD40 al inhibir la recombinación de cambio de clase de IgE y disminuir el número de linfocitos B secretores de IgE in vitro. Asimismo, tanto el sulfato de celulosa como fucoidán suprimieron la producción de IL-4 y potenciaron la producción de IFN-γ por parte de linfocitos T murinos estimulados con anticuerpos anti-CD3 y anti-CD28, pese a la disminución de la expresión de ARNm T-bet.
Conclusiones: La celulosa adquiere un efecto antialérgico en los linfocitos B y T al añadir grupos sulfato.

Palabras clave: Sulfatación de celulosa. Linfocitos T. Linfocitos B. IgE. Recombinación de cambio de clase.

Introduction

Immunoglobulin (Ig) E plays an important role in the onset and development of several allergic diseases. IgE binds to the high-affinity IgE receptor FcεRI on mast cells and basophils, and cross-linking of FcεRI by IgE and specific antigens induces the release of proinflammatory mediators, such as histamine, leukotrienes, and various cytokines [1]. The serum concentration of IgE is correlated with the level of FcεRI expression on the surface of basophils and mast cells [1-3] and with disease severity, especially that of cutaneous lesions [4,5]. Thus, controlling IgE production could be a promising therapeutic option for allergic diseases. In fact, a recombinant humanized anti-IgE monoclonal antibody, omalizumab, recently proved highly effective in the treatment of severe allergic asthma [6].

We previously reported that fucoidan, a dietary fiber extracted from seaweed, has an inhibitory effect on IgE production by preventing NF-κB p52 translocation in murine B cells isolated from spleen in vitro [7] and that peritoneal injection of fucoidan suppresses the increase in total and ovalbumin-specific IgE in mouse plasma induced by ovalbumin sensitization in vivo [8]. Fucoidan is composed of α1→3–linked L-fucose with a sulfate group at the 4 position on some of the fucose residues [9] and shows antiviral and antineoplastic activity. Several reports have suggested that these biological effects are associated with sulfate groups of sulfated polysaccharides [10,11]. Therefore, we hypothesized that neutral polysaccharides, such as cellulose, which is not water-soluble and does not have any natural antiallergic properties, could exhibit antiallergic effects when they adopt sulfate groups. Cellulose is the most common organic polymer produced by plants. It has a long single-chain structure and can therefore be readily sulfated and obtained in a uniform molecular structure.

We chemically prepared cellulose sulfate and investigated its activity in suppressing IgE production in murine B cells stimulated by interleukin (IL) 4 and anti-CD40 antibody in vitro. We evaluated degrees of IgE class-switching and populations of IgE-secreting B cells using ELISpot assay and measurement of the expression of Cε germline transcript. We also investigated the effects of cellulose sulfate on the production of IL-4 and inteferon (IFN) γ by T cells and the expression of mRNA by T-bet, a potent transcription factor for IFN-γ.

Materials and Methods

Preparation of Cellulose Sulfate

Cellulose sulfate was prepared according to the method described in previous reports [12,13], with slight modifications. Briefly, 50 mg of cellulose powder (ADVANTEC) was suspended in 400 μL of N,N-dimethylformamide (DMF) (SIGMA-Aldrich Japan) by stirring at room temperature for 1 hour, mixed with 98 mg of N,N-DMF sulfurtrioxide (SIGMA-Aldrich Japan), dissolved in 600 μL of DMF, and stirred overnight at 4°C to generate cellulose sulfate. The cellulose sulfate generated was precipitated by adding NaCl-saturated acetone and resolubilized in 5 mL of 10 mM-phosphate buffer (pH 7.4). pH was adjusted to 7.0 by adding 1M-phosphate buffer (pH 7.4), and the solution was purified by gel filtration using a Sephacryl S-300 column (GE Healthcare Japan). Amounts of total sugar and sulfate groups were measured using the phenol-sulfuric acid colorimetric method and Dodgson-Price turbidimetry [14], with potassium sulfate as a standard.

Isolation of Murine Spleen Cells, T Cells, and B Cells

Male 8-week-old BALB/c mice (Charles River Japan) were sacrificed, and the spleens were resected according to the Guidelines for Animal Experiments of Hiroshima University and the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University. The spleens were minced in Iscove's Modified Dulbecco's Medium (IMDM) (Invitrogen) containing 10% fetal calf serum and antibiotics (complete IMDM). The cells were cleaned by passage through a nylon mesh filter (70 μm) (BD Biosciences), and splenic lymphocytes (spleen cells) were collected by density separation with Lympholyte-M (Cedarlane Laboratories). Murine splenic B cells were isolated as previously described [15]. In brief, splenic lymphocytes were incubated with anti-CD43–coated metal microbeads (Miltenyi Biotec) and applied to an Auto-MACS column system (Miltenyi Biotec) according to the manufacturer's instructions. The unbound cells, of which more than 97% were CD19-positive, were collected as B cells. Likewise, murine splenic T cells were purified using the Pan T cells Isolation Kit II (Miltenyi Biotec).

Cell Culture

Splenic lymphocytes and isolated B cells (see above) were washed and resuspended in complete IMDM supplemented with 100 ng/mL of mouse IL-4 (R&D Systems) and 10 μg/mL of antimouse CD40 antibody (R&D). Each group of cell preparations was distributed into 96-well tissue culture plates (BD Biosciences) at 4×10^5 cells/well and maintained at 37°C in a 5% CO_2 atmosphere with vehicle alone, 100 μg/mL of fucoidan (Tanglewood), 100 μg/mL of methylcellulose (SIGMA-Aldrich, 15 cP), or 100 μg/mL of cellulose sulfate. The cultivation period was 4 days for the cell proliferation assay and measurement of Cε germline transcription levels, 5 days for the ELISpot assay, and 7 days for the measurement of IgE in culture supernatants. The isolated T cells (see above) were washed and resuspended in complete IMDM, distributed into 96-well tissue culture plates (BD Biosciences) at 2×10^5 cells/well, and preincubated at 37°C for 30 minutes in a 5% CO_2 atmosphere with vehicle alone, 100 μg/mL of fucoidan, 100 μg/mL of methyl cellulose, 100 μg/mL of cellulose sulfate, or 1 ng/mL of IFN-γ. They were then stimulated with 4 μg/mL of antimouse CD3 antibody (BD Biosciences) and 4 μg/mL of antimouse CD28 antibody (BD Biosciences), followed by culture for 3 days for the measurements of IL-4 and IFN-γ in supernatants or cultures for 24 hours for the measurement of T-bet mRNA levels by real-time reverse transcriptase polymerase chain reaction (RT-PCR).

Cell Proliferation Assay

Cell proliferation was measured using the CellTiter 96 Aqueous One Solution Proliferation Assay Kit (Promega), which is based on the cellular conversion of a tetrazolium salt into a soluble formazan product as a measure of proliferation, according to the manufacturer's instructions. Briefly, after incubation of B cells for 4 days or T cells for 3 days (see above), cells were further incubated with the Aqueous One solution reagent for 4 hours. Color intensity was measured at 490 nm using a 96-well plate reader.

Measurement of IgE, IL-4, and IFN-γ

The concentrations of IgE, IL-4, and IFN-γ in cell culture supernatant were determined using the BD OptEIA Mouse IgE ELISA Set (BD Biosciences), BD OptEIA Mouse IL-4 ELISA Set (BD Biosciences), and Quantikine Mouse IFN-γ Immunoassay (R&D Systems), respectively, following the manufacturers' instructions.

Measurement of Cε Germline Transcription Levels by Real-time RT-PCR

Total RNA was extracted from B cells using the RNeasy Mini Kit (Qiagen), and cDNA was generated using the QuantiTect Reverse Transcription kit (Qiagen). Levels of Cε germline transcription were evaluated using the ABI 7300 Real-time PCR System (Applied Biosystems) with Power SYBR Green PCR Master Mix (Applied Biosystems). The specific primer pairs were as follows: forward primer for ε germline transcripts, 5′-GCA CAG GGG GCA GAA GAT-3′; reverse primer for ε germline transcripts, 5′-CGT TGA ATG ATG GAG GAT-3′; forward primer for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-AAC GAC CCC CTT CAT TGA C-3′; reverse primer for GAPDH, 5′-TCC ACG ACA TAC TCA GCA C-3′. Expression of GAPDH was measured as an internal control to calibrate gene expression.

ELISpot Assay

The number of IgE-secreting B cells was determined using the ELISpot assay (Protein Detector AP ELISpot kit, KPL) following the manufacturer's instructions. The preincubated B cells were cultured overnight in an ELISpot 96-well plate coated with 15 μg/mL of antimouse IgE antibody (R35-72) (BD). The released IgE captured on the plate was then stained with 0.5 μg/mL of biotin-conjugated antimouse IgE antibody (R35-118) (BD), alkaline phosphatase-conjugated streptavidin, and the substrate of alkaline phosphatase-conjugated streptavidin. Numbers of spots were counted manually under a stereomicroscope.

Measurement of T-bet mRNA by Real-time RT-PCR

Total RNA was extracted from T cells and converted to cDNA as described above for B cells. The amount of T-bet mRNA was evaluated as described above for Cε germline transcription. The specific primer pairs for T-bet were as follows: forward primer, 5′-GCC AGG GAA CCG CTT ATA TG-3′; reverse primer, 5′-GAC GAT CAT CTG GGT CAC ATT-3′.

Statistics

Statistical analysis was performed using the Mann-Whitney test. A P value of <.05 was considered significant.

Results

Cellulose Sulfate and Cytotoxic Activity

Natural cellulose powder was chemically sulfated and fractionated by gel filtration as described in Materials and Methods. Based on the findings of our previous study on hydrolysed konjac glucomannan [16], we collected fractions of around 10 kDa (Figure 1), which were then freeze-dried and used as cellulose sulfate in further experiments. The concentration of sulfate groups in the preparation of cellulose sulfate was 0.26 ± 0.02 mg (mean ± SD) per 1 mg of total sugar.

The effect of cellulose sulfate on lymphocyte proliferation and survival was examined using B cells and T cells. As shown in Figure 2, the amount of formazan product, which reflects cell proliferation, was not suppressed by fucoidan, methylcellulose, or cellulose sulfate. Bromodeoxyuridine (BrdU) chemiluminescent assay did not reveal cytotoxicity (data not shown).

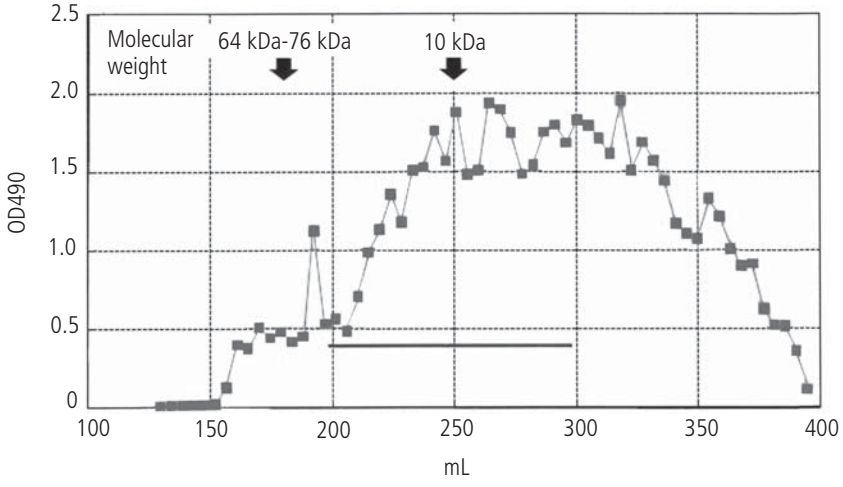


Figure 1. Purification of cellulose sulfate. Cellulose sulfate was generated (see Materials and Methods) and purified by gel filtration with a Sephacryl S-300 column. Elution volume fractions from 200 to 300 mL were collected and freeze-dried. Compared with dextran molecular weight markers, the molecular weight of the cellulose sulfate was around 10 kDa.

Cellulose Sulfate Suppresses IgE Production From Spleen Cells and B Cells In Vitro

Spleen cells and purified B cells were cultured with IL-4 and anti-CD40 antibody in the presence or absence of the

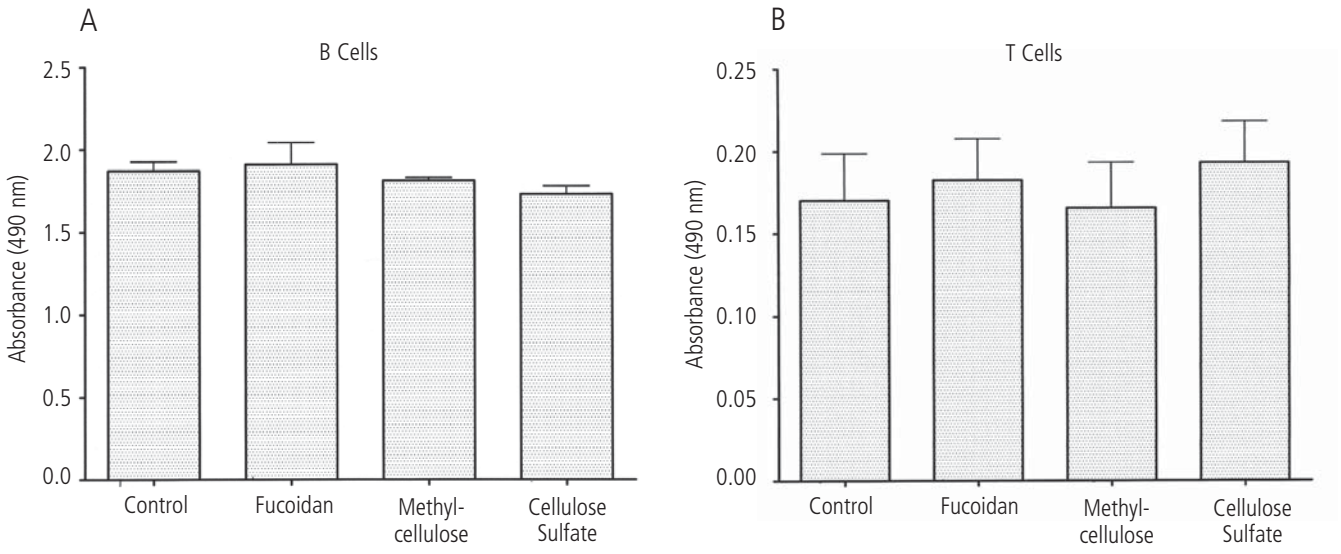
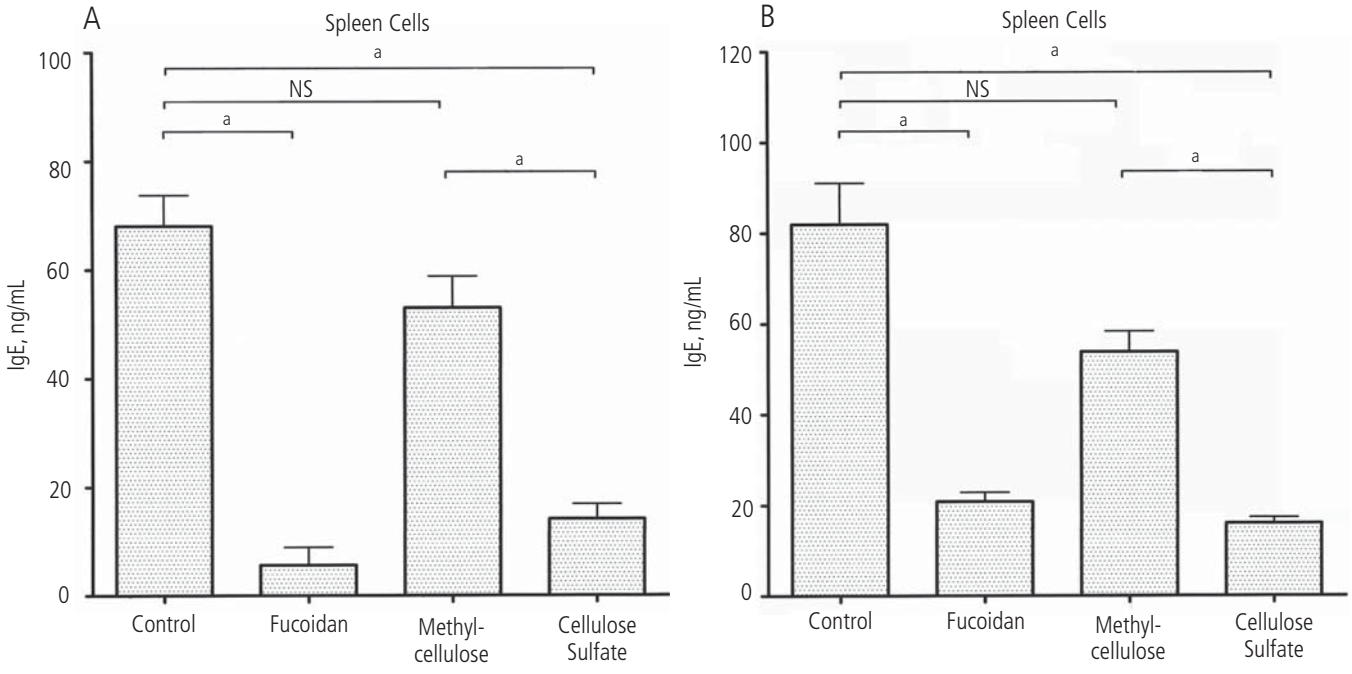


Figure 2. Sulfated cellulose does not show cytotoxicity against murine lymphocytes. A, B cells derived from spleen cells of BALB/c mice were cultured for 4 days with interleukin 4 (100 ng/mL) and anti-CD40 antibody (10 μg/mL) in the presence or absence of fucoidan (100 μg/mL), methylcellulose (100 μg/mL), or cellulose sulfate (100 μg/mL). B, T cells derived from spleen cells were cultured for 3 days with anti-CD3 antibody (4 μg/mL) and anti-CD28 antibody (4 μg/mL) in the presence or absence of the polysaccharides. The lymphocytes were mixed with tetrazolium salt solution, and the absorbance of soluble formazan products was measured at 490 nm. Data were expressed as the mean (SEM) of 1 representative experiment performed in quadruplicate. Similar data were obtained in 2 independent experiments.



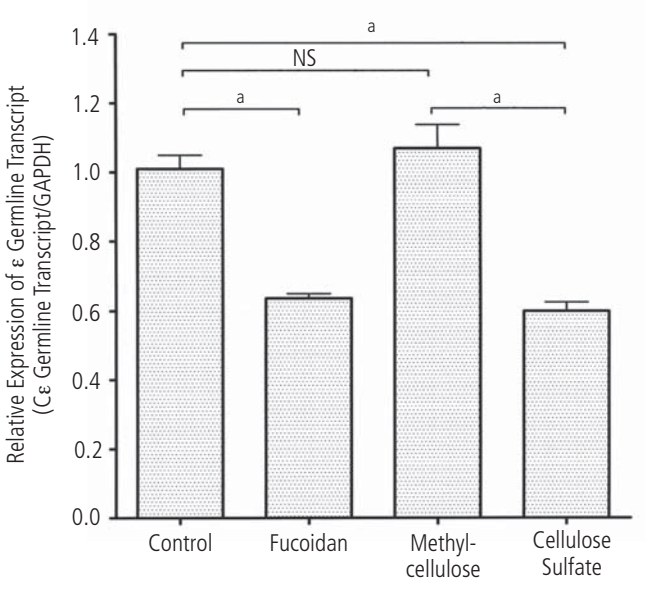
[a]$P<.05$ (significantly different from control by the Mann-Whitney test).

Figure 3. Cellulose sulfate suppresses IgE production of spleen cells and B cells. Spleen cells (A) and purified B cells (B) were cultured with mouse interleukin 4 (100 ng/mL) and antimouse CD40 antibody (10 μg/mL) in the presence or absence of fucoidan (100 μg/mL), methylcellulose (100 μg/mL), or cellulose sulfate (100 μg/mL) for 7 days. Immunoglobulin E concentrations in culture supernatants were measured using enzyme-linked immunosorbent assay. Data were expressed as the mean (SEM) of 1 representative experiment performed in quadruplicate. Similar results were obtained in 2 independent experiments. Ig indicates immunoglobulin; NS, nonsignificant.

polysaccharides for 7 days. As shown in Figure 3, cellulose sulfate markedly inhibited the increase in IgE production induced by IL-4 and anti-CD40 antibody, as did fucoidan. Methylcellulose, which was water-soluble and was used as a counterpart of cellulose sulfate, was unable to suppress IgE production in spleen cells and B cells.

Cellulose Sulfate Suppresses Expression of Cε Germline Transcript in B Cells

To investigate the effect of cellulose sulfate on IgE class-switching, we measured levels of Cε germline transcription in B cells, which were cultured with IL-4 and anti-CD40 antibody in the presence or absence of polysaccharides for 4 days. Cellulose sulfate significantly inhibited the induction of Cε germline transcription as fucoidan, but no effect was observed in the presence of the same amount of methylcellulose (Figure 4). This result suggested that IgE production suppressed by cellulose sulfate was due, at least in part, to de novo prevention of IgE class-switching in B cells.

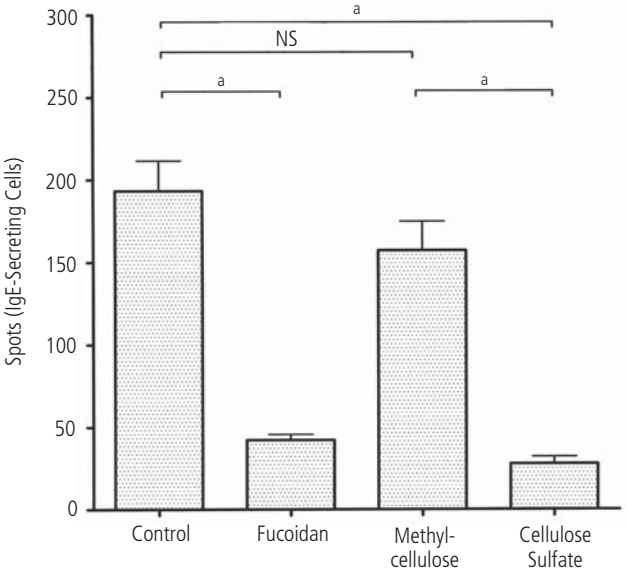


[a]*P*<.05 (significantly different from control by the Mann-Whitney test).

Figure 4. Cellulose sulfate suppresses the production of Cε germline transcript. B cells derived from spleen cells of BALB/c mouse were cultured with mouse interleukin 4 (100 ng/mL) and antimouse CD40 antibody (10 μg/mL) in the presence or absence of fucoidan (100 μg/mL), methylcellulose (100 μg/mL), or cellulose sulfate (100 μg/mL) for 4 days. The transcriptional levels of Cε germline were measured by real-time reverse transcriptase polymerized chain reaction normalized by glyceraldehyde-3-phosphate dehydrogenase. Data were expressed as the mean (SEM) of the ratio relative to the control in 1 representative experiment performed in quadruplicate. Similar results were obtained in 2 independent experiments. GAPDH, indicates glyceraldehyde-3-phosphate dehydrogenase; NS, nonsignificant.

Cellulose Sulfate Decreases the Number of IgE-Secreting B Cells

To further examine the effect of sulfated cellulose on B-cell differentiation toward the IgE-secreting phenotype, we performed ELISpot assay for IgE. Spots on the membranes indicate a number of IgE-secreting B cells. Both cellulose sulfate and fucoidan significantly suppressed the increase in IgE-secreting B cells (Figure 5).

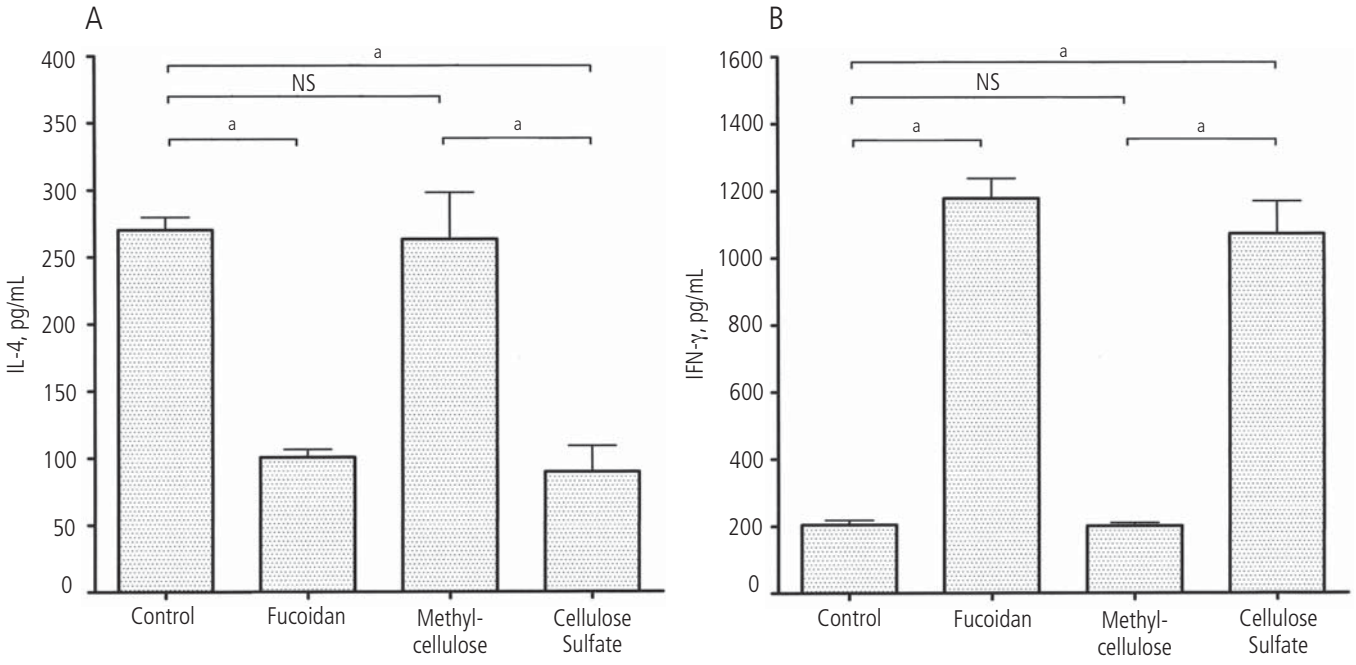


[a]*P*<.05 (significantly different from control by the Mann-Whitney test).

Figure 5. Cellulose sulfate reduces the number of IgE-secreting B cells. B cells derived from spleen cells of BALB/c mice were cultured with mouse interleukin 4 (100 ng/mL) and antimouse CD40 antibody (10 μg/mL) in the presence or absence of fucoidan (100 μg/mL), methylcellulose (100 μg/mL), or cellulose sulfate (100 μg/mL) for 5 days. The cells were then cultured overnight on a plate coated with capture antibody. Released immunoglobulin E bound to the membrane was stained with biotinylated antimouse antibody, AP-streptavidin, and BCIP/NBT substrate. The number of spots was counted under a stereomicroscope. Data were expressed as the mean (SEM) of 1 representative experiment performed in quadruplicate. Similar data were obtained in 2 independent experiments. Ig indicates immunoglobulin; NS, nonsignificant.

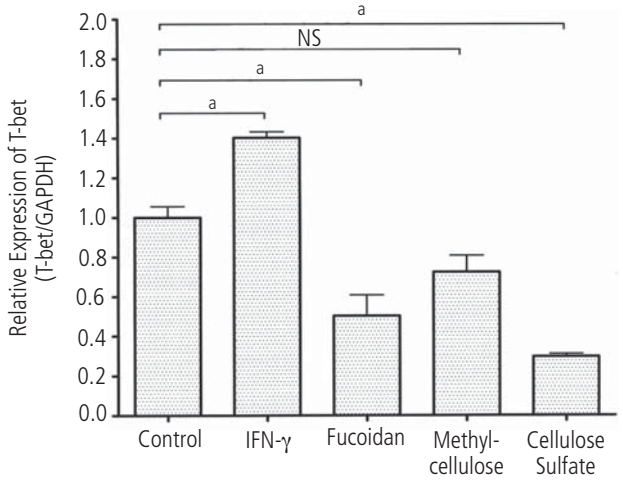
Cellulose Sulfate Affects Cytokine Production of T Cells

To explore the possibility of an indirect effect of cellulose sulfate on B cells through T cells, we investigated the effect of cellulose sulfate on production of type 1 helper T cells (T_H1) and T_H2. T cells purified from murine spleen were stimulated with anti-CD3 and anti-CD28 antibodies in the presence or absence of fucoidan, methylcellulose, and cellulose sulfate for 3 days. Both cellulose sulfate and fucoidan significantly suppressed the production of IL-4, a representative T_H2 cytokine, from T cells (Figure 6). In contrast, the production of IFN-γ, a representative T_H1 cytokine, was significantly enhanced in the presence of cellulose sulfate and of fucoidan (Figure 6).



[a]*P*<.05 (significantly different from control by the Mann-Whitney test).

Figure 6. Cellulose sulfate suppresses IL-4 and enhances IFN-γ production by T cells derived from splenic cells. T cells were preincubated with vehicle alone, fucoidan (100 µg/mL), methylcellulose (100 µg/mL), or cellulose sulfate (100 µg/mL) before being cultured with anti-CD3 antibody (4 µg/mL) and anti-CD28 antibody (4 µg/mL) for 3 days. IL-4 (A) and IFN-γ (B) concentrations in culture supernatants were measured using enzyme-linked immunosorbent assay. Data were expressed as the mean (SEM) of 1 representative experiment performed in quadruplicate. Similar data were obtained in 2 independent experiments. IL indicates interleukin; IFN, interferon; NS, nonsignificant.



[a]*P*<.05 (significantly different from control by the Mann-Whitney test).

Figure 7. Cellulose sulfate downregulates the expression of T-bet mRNA. T cells were preincubated with vehicle alone, fucoidan (100 µg/mL), methylcellulose (100 µg/mL), cellulose sulfate (100 µg/mL), or interferon γ (1 ng/mL) for 30 minutes before being stimulated with antimouse CD3 antibody (4 µg/mL) and antimouse CD28 antibody (4 µg/mL) for 24 hours. T-bet mRNA levels were measured by real-time reverse transcriptase polymerase chain reaction, normalized by GAPDH, and expressed as the mean (SEM) of the ratio relative to the control in 1 representative experiment performed in quadruplicate. Similar results were obtained in 2 independent experiments. GAPDH indicates glyceraldehyde-3-phosphate dehydrogenase; NS, nonsignificant.

Cellulose Sulfate Downregulates Expression of T-bet mRNA

In order to further investigate the mechanism of T_H1/T_H2 balance regulation by cellulose sulfate, we measured levels of T-bet mRNA expression using real-time RT-PCR. The expression of T-bet mRNA was downregulated by both cellulose sulfate and fucoidan, whereas it was upregulated by IFN-γ (Figure 7).

Discussion

In the present study, we showed that cellulose sulfate and fucoidan suppressed IgE production from spleen cells and purified B cells stimulated with IL-4 and anti-CD40 antibody in vitro. Cellulose sulfate also inhibited expression of Cε germline transcript and the increase in IgE-secreting B cells. Moreover, we demonstrated that cellulose sulfate suppresses IL-4 production and enhances IFN-γ production from purified T cells stimulated with anti-CD3 and anti-CD28 antibodies.

Cellulose is a neutral homopolysaccharide composed of glycosidically bound ß-1,4 D-glucopyranose, which is produced from water and carbon dioxide by photosynthesis of plants. It is poorly water-soluble, chemically stable, and accounts for most of the dry weight of plant body. Therefore, it is the most common organic polymer, representing about 1.5×10^{12} tons of total annual biomass production [17].

The particular physiological activity of cellulose in living organisms is unknown. Methylcellulose, which is water soluble and commonly used in cell culture, did not show any marked biological effects in our study. In contrast, cellulose attached by sulfate groups (cellulose sulfate) gained de novo antiallergic properties. Our findings support the hypothesis that sulfate groups might be crucial to achieving various biological functions in sulfated polysaccharides. Yang et al [18] reported that the degree of sulfation (DS), molecular weight (MW), and branching structure of Chinese lacquer polysaccharide were related to anticoagulant activity. These authors concluded that polysaccharides with a DS of 1.15 and the highest MW of 34.9 kDa had the best anticoagulant activity. However, the cellulose sulfate generated in the present study markedly suppressed IgE with a substantially smaller DS (0.66) and MW (around 10 kDa) than the conditions used by Yang et al. Thus, different biological functions of polysaccharides may require different DS and MW as their optional conditions.

IgE synthesis is regulated not only at the level of B cells, but also at that of T cells, especially via regulation of the T_H1 and T_H2 balance. The representative T_H1 cytokine, IFN-γ, inhibits differentiation of T_H2 cells, decreases production of IL-4, and leads to inhibition of IgE synthesis by B cells. On the other hand, T_H2 cytokines, such as IL-4 and IL-13, induce expression of the Cε germline transcript in B cells and promote class-switch recombination to IgE in concert with CD40 ligands [19]. Our results indicate that cellulose sulfate induced a T_H1 shift in T cells and that this effect was also dependent on the sulfate groups of polysaccharides.

The downregulation of T-bet mRNA observed in this study (Figure 7) was unexpected, since T-bet is a member of the T-box family, which induces expression of IFN-γ, a hallmark T_H1 cytokine [20]. Moreover, the expression of T-bet itself is also enhanced by positive feedback from IFN-γ [21]. However, Usui et al [22] demonstrated that T-bet–deficient cells incubated with IL-12 and anti-IL-4 antibody (strict T_H1–inducing condition) were almost normally differentiated to T_H1 cells. Therefore, T-bet is not necessarily essential for production of IFN-γ. Usui et al [23] also revealed that the activation of the STAT4 pathway induced by IL-12 may drive T cells to differentiate towards the T_H2 phenotype. Yang et al [24] showed that the activation of NF-κB by IL-18 induced expression of IFN-γ mRNA through GADD45ß. Our findings suggest that cellulose sulfate induces the T_H1-dominant condition via a pathway that does not depend on T-bet and suppresses positive feedback by IFN-γ on T-bet production. Further studies concerning the effect of cellulose sulfate on these T cell transcription factors and related cytokines are necessary to elucidate the entire mechanism for suppressing IgE production by cellulose sulfate and presumably by other sulfated polysaccharides, such as fucoidan.

In conclusion, cellulose sulfate inhibited IgE production through suppression of IgE class-switching in B cells in vitro. Cellulose sulfate also induced a shift in the T_H1/T_H2 balance toward the T_H1-dominant condition. Further studies should be undertaken to clarify the molecular mechanism (eg, receptors of cellulose sulfate and fucoidan) and subsequent signaling molecules affecting IgE production of B cells and T cells, as well as the cell-to-cell interactions affected by these polysaccharides. Chemical or enzymatic modifications of cellulose sulfate should facilitate studies of this type, thanks to their simple nonbranching structure, a common feature of many polysaccharides, including fucoidan.

Acknowledgments

We thank Dr Faiz Kermani for a critical review of the manuscript. This work was carried out at the Analysis Center of Life Science, Hiroshima University.

This study did not receive external financial support. The authors report no conflicts of interest.

References

1. Holgate ST. The role of mast cells and basophils in inflammation. Clin Exp Allergy. 2000;30 Suppl 1:28-32.
2. MacGlashan DW Jr, Schroeder JT. Functional consequences of FcεRIα up-regulation by IgE in human basophils. J Leukoc Biol. 2000;68:479-86.
3. Saini SS, MacGlashan DW Jr,Sterbinsky SA, Togias A, Adelman DC, Lichtenstein LM, Bochner BS. Down-regulation of human basophil IgE and FC epsilon RI alpha surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo. J Immunol. 1999;162:5624-30.
4. Laske N, Niggemann B. Does the severity of atopic dermatitis correlate with serum IgE levels? Pediatr Allergy Immunol. 2004;15:86-8.
5. Ahmed I, Nasreen S. Frequency of raised serum IgE level in childhood atopic dermatitis. J Pak Med Assoc. 2007;57:431-4.
6. Busse W, Corren J, Lanier BQ, McAlary M, Fowler-Taylor A, Cioppa GD, van As A, Gupta N. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. J Allergy Clin Immunol. 2001;108:184-90.
7. Oomizu S, Yanase Y, Suzuki H, Kameyoshi Y, Hide M. Fucoidan prevents Cε germline transcription and NFκB p52 translocation for IgE production in B cells. Biochem Biophys Res Commun. 2006;350:501-7.
8. Yanase Y, Hiragun T, Uchida K, Ishii K, Oomizu S, Suzuki H, Mihara S, Iwamoto K, Matsuo H, Onishi N, Kameyoshi Y, Hide M. Peritoneal injection of fucoidan suppresses the increase of plasma IgE induced by OVA-sensitization. Biochem Biophys Res Commun. 2009;387:435-9.
9. Patankar MS, Oehninger S, Barnett T, Williams RL, Clark GF. A revised structure for fucoidan may explain some of its biological activities. J Biol Chem. 1993;268:21770-6.
10. Liu JM, Haroun-Bouhedja F, Boisson-Vidal C. Analysis of the in vitro inhibition of mammary adenocarcinoma cell adhesion by sulfated polysaccharides. Anticancer Res. 2000;20:3265-71.
11. Schaeffer DJ, Krylov VS. Anti-HIV activity of extracts and compounds from algae and cyanobacteria. Ecotoxicol Environ Saf. 2000;45:208-27.
12. Leali D, Belleri M, Urbinati C, Coltrini D, Oreste P, Zoppetti G, Ribatti D, Rusnati M, Presta M. Fibroblast growth factor-2 antagonist activity and angiostatic capacity of sulfated Escherichia coli K5 polysaccharide derivatives. J Biol Chem. 2001;276:37900-8.

13. Schweiger RG. Polysaccharide sulfates. I. Cellulose sulfate with a high degree of substitution. Carbohydr Res. 1972;21:219-28.
14. Dodgson KS, Price RG. A note on the determination of the ester sulphate content of sulphated polysaccharides. Biochem J. 1962;84:106-10.
15. Morimoto K, Nabeshima Y, Mochizuki M, Oomizu S, Yamamoto S, Hide M. The extract of syngeneic keratinocytes enhances IgE production from BALB/c mouse splenic lymphocytes in vitro. Arch Dermatol Res. 2006;297:358-66.
16. Suzuki H, Oomizu S, Yanase Y, Onishi N, Uchida K, Mihara S, Ono K, Kameyoshi Y, Hide M. Hydrolyzed konjac glucomannan suppresses IgE production in mice B cells. Int Arch Allergy Immunol. 2010;152:122-30.
17. Klemm D, Heublein B, Fink HP, Bohn A. Cellulose: fascinating biopolymer and sustainable Raw Material. Angew Chem Int Ed Engl. 2005;44:3358-93.
18. Yang J, Du Y, Huang R, Wan Y, Li T. Chemical modification, characterization and structure-anticoagulant activity relationships of Chinese lacquer polysaccharides. Int J Biol Macromol. 2002;31:55-62.
19. Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class-switch recombination. Nat Rev Immunol. 2003;3:721-32.
20. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000;100:655-69.
21. Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, Nguyen BV, Gadina M, Sher A, Paul WE, O'Shea JJ. T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. Proc Natl Acad Sci U S A. 2001;98:15137-42.
22. Usui T, Preiss JC, Kanno Y, Yao ZJ, Bream JH, O'Shea JJ, Strober W. T-bet regulates Th1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. J Exp Med. 2006;203:755-66.
23. Usui T, Nishikomori R, Kitani A, Strober W. GATA3 suppresses Th1 development by downregulation of Stat4 and not through effects on IL-12Rbeta2 chain or T-bet. Immunity. 2003;18:415-28.
24. Yang J, Zhu H, Murphy TL, Ouyang W, Murphy KM. IL-18-stimulated GADD45 beta required in cytokine-induced, but not TCR-induced, IFN-gamma production. Nat Immunol. 2001;2:157-64.

Manuscript received August 17, 2011; accepted for publication November 25, 2011

Michihiro Hide, MD, PhD

Department of Dermatology
Division of Molecular Medical Science
Graduate School of Biomedical Sciences,
Hiroshima University
1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan
E-mail: ed1h-w1de-road@hiroshima-u.ac.jp