

Anti-Allergic Effect of Soluble Beta-Glucan on Japanese Cedar Pollinosis and Mechanism Underlying Its Allergy-Ameliorating Action

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Abstract

Background: Japanese cedar (sugi) pollinosis is triggered by sensitization to sugi pollen, which contains sugi antigens, sugi basic proteins (SBP: Cryj1 and Cryj2), and adjuvant polysaccharides, including insoluble β -glucan particles (IBGN: a dectin-1 agonist). The presence of IBGN in sugi pollen enhances the production of SBP-specific IgE, which contributes to the inflammatory response through mast cell activation, leading to degranulation and resulting in symptoms such as sneezing. Another β -glucan derived from black yeast, soluble β -glucan (SBGN: a dectin-1 antagonist), is recognized for its anti-allergic properties. **Objective:** This study aimed to elucidate the anti-allergic effects of SBGN and to uncover the mechanisms underlying its anti-allergic action in a mouse model of sugi pollinosis. **Methods:** Mice were utilized in both studies, focusing on the pollinosis model and gut microbiota. The study on pollinosis comprised three groups: a control group (Ctr), a pollinosis group (Polli), and an SBGN-administered pollinosis group (SBGN + Polli). A DNA microarray was employed to uncover molecular events associated with SBGN-mediated anti-allergic action using RNA extracted from the iliac lymph nodes of the three groups. The gut microbiota was analyzed using real-time PCR on DNA samples from the two groups. **Results:** Oral administration of SBGN suppressed both SBP-specific IgE production and SBP-induced sneezing in the sugi pollinosis mouse model. DNA microarray analyses revealed that SBGN mitigated the dysregulated expression of 19 immune-inflammatory genes, including Igk and Chi3l3, which were abnormally up- or down-regulated in the iliac lymph nodes of mice with sugi pollinosis. Furthermore, SBGN administration affected gut microbiota by increasing probiotic bacteria such as bifidobacteria,

which are known to produce short-chain fatty acids (SCFAs). **Conclusion:** SBGN exhibited anti-allergic effects against sugi pollinosis by inhibiting the immune response (SBP-induced IgE production) and by reducing the inflammatory reaction (SBP-induced sneezing). Additionally, SBGN was shown to increase SCFA-producing gut bacteria, thereby suggesting a role in regulating mast cell activation.

Keywords

Functional Foods, Pollinosis, Dectin-1, DNA Microarray

1. Introduction

β -glucans are naturally occurring polysaccharides of D-glucose monomers linked by β -glycosidic bonds, which feature a β -1,3-glucan backbone with diverse branching structures. β -glucans exhibit well-defined structure-activity relationships; differences across their various sources and structures result in distinct biological activities. The physicochemical characteristics of β -glucans can differ, such as some may be insoluble, form aggregates, and have high molecular weight, whereas others may be soluble, do not form aggregates, and have low molecular weight. These differences in physicochemical properties are critical and influence different biological activities, including immunomodulation [1]. As β -glucans are exogenous compounds, such as pathogens, for mammals, including rodents and primates, they are recognized by pattern recognition receptors (PRRs) to evoke intracellular β -glucan signaling machinery [1] [2]. Dectin-1 is a type II C-lectin-like membrane receptor of the PRR family and is expressed on the surface of myeloid cells (including lymphocytes, macrophages, neutrophils, and mast cells). β -glucans bind to the carbohydrate recognition domain of dectin-1. Notably, insoluble β -glucan particles (IBGN) are able to induce oligomerization and elicit dectin-1 signaling, resulting in Src activation and Syk recruitment, but soluble β -glucans (SBGN) are unable to do these reactions [1] [3] [4]. IBGN stimulates an immune-inflammatory response that alters gene expression, cytokine and chemokine production, antigen presentation to T and B cells, and immunoglobulin production [1]. Conversely, SBGN inhibits the stimulatory actions of IBGN by competitively binding to dectin-1, thereby acting as a dectin-1 antagonist.

Pollen allergy is an IgE-mediated type I hypersensitivity reaction in which the production of antigen-specific IgE antibodies and the activation of mast cells are the limiting factors. Inclination toward a type 2 immune state, Th2, promotes the production of IgE antibodies directed toward a specific antigen called an allergen. IgE antibodies bind to high-affinity IgE receptors (Fc ϵ RI) on mast cells and basophils, activating them to trigger degranulation for the release of varied inflammatory mediators, including histamine.

Pollen is not only an allergen carrier but also an adjuvant during the induction phase of the allergic immune response [5]. Callose is a major component of higher

plant pollen and has a polysaccharide nature with β -1,3-glucan [6] [7]. Pollen from Japanese cedar, known as sugi, contained dectin-1 reactive β -glucan [8], which induces the production of sugi-allergen-specific IgE in mice [9]. The C-type lectin receptor, dectin-1, was known to be involved in the polarization of CD4⁺ T cells towards specific Th2 responses associated with specific IgE production and allergic diseases [10]. In a previous paper it was reported that sugi pollen contained IBGNs, which had a potent adjuvant activity via binding to dectin-1 on both antigen-presenting and lymphoid cells, leading to an allergic response to produce a specific IgE antibody towards sugi allergen, sugi basic protein (SBP), Crj-1 and Crj-2 [11].

SBGN is another type of β -glucan with β -1,6-branched structures and is mainly produced by the black yeast, *Aureobasidium pullulans* [12]. The SBGN obtained from black yeasts is recognized for its immunoregulatory properties, which can alleviate allergic and/or inflammatory disorders [12] [13]. SBGNs and their related compounds function as dectin-1 antagonists and are gaining attention for use as anti-allergic and anti-inflammatory materials. However, detailed information on the mechanism underlying SBGN-mediated anti-allergic action has not been sufficiently accumulated yet.

In this study, we aimed to examine the anti-allergic effects of SBGN and the inhibition of both SBP-specific IgE production and SBP-induced sneezing in a mouse model of sugi-pollinosis. We also aimed to elucidate the mechanism underlying the SBGN-evoked anti-allergic action using a DNA microarray, yielding previously unknown molecular events that occurred in the iliac lymph nodes of the pollinosis model.

2. Materials and Methods

2.1. Reagents

The following materials were used in this study: phosphate-buffered saline (PBS) (Nissui, Tokyo, Japan), a Nunc-immunomodule, F8 Maxisorp, streptavidin-horseradish peroxidase (SA-HRP) (Thermo Fisher Scientific K.K., Yokohama, Japan), and tetramethylbenzidine (TMB) (Sigma-Aldrich Co. LLC, Tokyo, Japan). Casein Na, sodium azide (NaN₃), and Tween 20 were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). An ELAST enzyme-linked immunosorbent assay (ELISA) amplification system (PerkinElmer, Inc., Waltham, MA, USA), anti-mouse IgE (ab11580; Abcam, Cambridge, UK), streptavidin-horseradish peroxidase conjugate (SA10001; Invitrogen, Carlsbad, CA, USA), and biotinyltyramide (PerkinElmer) were used.

Furthermore, the RNAiso Plus, PrimeScriptTM Reverse Transcriptase, Recombinant RNase Inhibitor, TBGreen. Premix Ex TaqTM (Tli RNaseH Plus), RR420 (Takara Bio Inc., Kyoto, Japan), RNeasy MinElute Cleanup Kit (QIAGEN, Tokyo, Japan), dNTP Mix and Oligo (dT)15 Primer (Promega, Tokyo, Japan), Low Input Quick Amp Labeling Kit, RNA6000 Nano Kit, Agilent Whole Human Genome DNA Microarray 4 × 44K v2, and Agilent Gene Expression Hybridization Kit (Ag-

ilent Technologies, Santa Clara, CA, USA) were also used in this study.

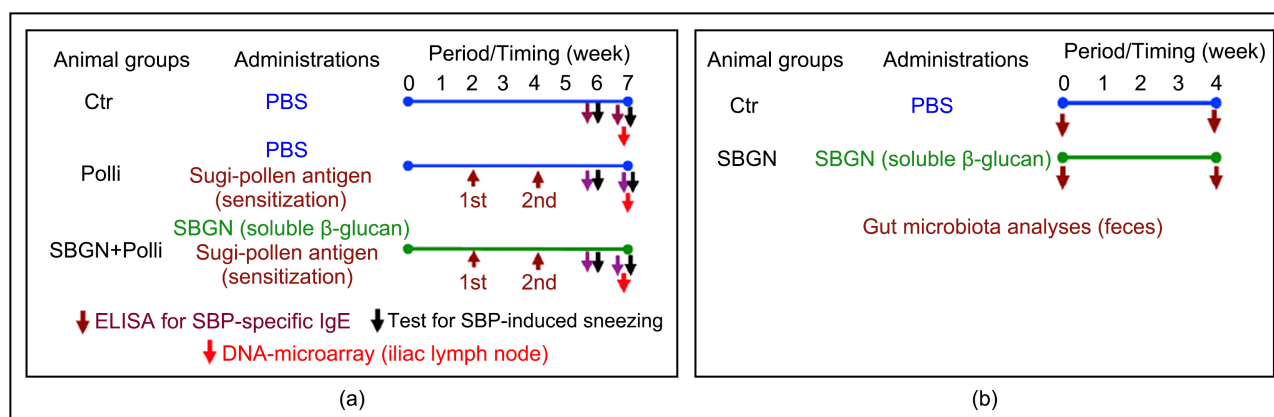
2.2. Preparation of Sugi-Pollen Suspension and Sugi-Basic Protein (SBP)

Japanese cedar (sugi) pollen (Biostir Inc., Osaka, Japan) was treated with ethanol, followed by preparation of a suspension with saline at a final concentration of 50 mg/ml. The obtained Sugi-pollen suspension was used for sensitization as a Sugi-pollen antigen to prepare a mouse model of pollinosis. Sugi-basic protein extract (SBP) was prepared by extracting the sugi pollen suspension with 0.9% NaCl containing 3% glycerol to yield a final concentration of 20 mg/mL Cryj-1 antigen. A quantitative assay for Cryj1 was performed using an LBIS Cryj-1 ELISA Kit (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

2.3. Preparation of Soluble Beta-Glucan (SBGN)

Crude SBGN was obtained from culture supernatants of *Aureobasidium pullulans* (ATCC No. 2052). Purified SBGN was prepared from the crude SBG by precipitation with 70% ethanol, followed by freeze-drying and dissolution in a sterile 0.9% NaCl solution. The amount the SBGN was assayed according to the POPOD procedure using the β -glucan Assay Kit (Neogen Japan, Yokohama, Japan). In this study, we used 0.1% (1 mg/ml) β -glucan solution.

2.4. Animals



(a) shows pollinosis examinations. The control group (Ctrl) and the pollinosis group (Polli) were orally given PBS for 7 weeks, respectively. The SBGN-administered pollinosis group (SBGN + Polli) was orally administered with SBGN for 7 weeks. Polli and SBGN + Polli groups were sensitized twice, 1st at 2 weeks and 2nd at 4 weeks, with sugi pollen antigen. ELISA for SBP-specific IgE and a test for SBP-induced sneezing were performed at both 4 and 6 weeks. DNA microarray was carried out at 6 weeks. (b) displays gut microbiota analyses using stool samples from two mouse groups, the control group (Ctrl) and the SBGN-administered group (SBGN). Ctrl and SBGN were orally administered with PBS and SBGN for 4 weeks, individually.

Figure 1. Outline for pollinosis examinations in mice and gut microbiota analyses.

Female BALB/c mice were used in both studies, focusing on the pollinosis model and gut microbiota. As illustrated in **Figure 1(a)**, the pollinosis model study comprised three groups: a control group (Ctrl) that received PBS for 7 weeks,

a pollinosis group (Polli) that was orally administered PBS for 7 weeks and sensitized twice with a sugi-pollen suspension at 2 and 4 weeks, and an SBGN-administered pollinosis group (SBGN + Polli) that received SBGN for 7 weeks and underwent the same sensitization with the sugi-pollen suspension at 2 and 4 weeks. **Figure 1(b)** outlines the timeline and examinations for the microbiota study, which involved two animal groups: a control group (Ctr) and SBGN group, which were orally administered PBS and SBGN for 4 weeks, respectively. In both studies, each group consisted of seven animals for the pollinosis study and six animals for microbiota examination.

2.5. Sensitization with Sugi-Pollen Antigen

Mice of the Polli and SBGN + Polli groups were sensitized twice: first at 2 weeks and then at 4 weeks. This was performed using intramuscular injections of a sugi-pollen suspension (100 μ L/mouse) at the right and left bases of the tail without any adjuvant (see **Figure 1(a)**). The sensitization procedure was conducted under ketamine hydrochloride anesthesia.

2.6. Preparation of Anti-SBP IgE Standard for ELISA

In preliminary studies, mice were sensitized with a sugi-pollen suspension, as mentioned above, to obtain anti-SBP sera for standard stock. Aliquots of the stocks were stored at -70°C until further use. The standard stock was serially diluted two-fold from 1:100 to 1:6400 to obtain a standard preparation with optical density from 1.8 to 0.1 at 450 nm using the enzyme-linked immunosorbent assay (ELISA). Arbitrary units were assigned to the standards for stable titration, with the least diluted standard (1:100) being 100 and the most diluted standard (1:6400) being 2.0. The correlation between the dilution and units was always >0.9 .

2.7. ELISA for SBP-Specific IgE

Blood samples were collected from the tail vein of each mouse at 6 and 7 weeks of the examination period (**Figure 1(a)**) using a hematocrit capillary tube pretreated with heparin and then centrifuged at $1000 \times g$ for 10 min to obtain plasma. Briefly, SBP (2 $\mu\text{g/mL}$ in PBS) was coated onto Immuno Module Maxisorp 96-well plates 4°C overnight. After blocking with 1% casein Na at 4°C overnight, standards and plasma samples, diluted 1:300 in 1% casein-Na prepared in PBS. Next, biotin-conjugated rat monoclonal anti-mouse IgE prepared at 0.8 $\mu\text{g/mL}$ in 1% casein Na - PBS was reacted followed by successive application of streptavidin-horse radish peroxidase conjugate, biotinyltyramide, and streptavidin-horseradish peroxidase. Color was developed by adding a colorimetric substrate (TMB). The enzyme reaction was terminated with 2M sulfuric acid and the plate was read at 450 nm by subtracting the reference determined at 630 nm using an iMark microplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA). The plates were washed with 0.05% Tween-20 in PBS between each step except when sulfuric acid was added. The antibody titers of the samples were calculated using a calibration curve gen-

erated using a serially diluted standard.

2.8. Test for SBP-Induced Sneezing

The mice were individually placed in observation cages, and their sneezing behavior was monitored using a video camera at 6 and 7 weeks of the examination period (**Figure 1(a)**). Both nasal cavities were pretreated with 10 μ L/site of N-acetylcysteine at a final concentration of 3 mg/mL to perturb the mucin layer [14]. After washing twice with PBS (10 mL/site of PBS), the mice were intranasally challenged with 10 mL/site of an allergen, SBP. For each observation session, the number of sneezes during the 5 min after intranasal challenge with SBP was counted by at least three investigators. The average number of sneezes per session was calculated. In cases where the coefficient of variation exceeded 10%, the sneezing behavior of the mouse was re-examined by viewing the recorded video again.

2.9. Gut Microbiota Analyses

Fresh stool samples were collected in the morning at 0 and 4 weeks during the experimental periods (**Figure 1(b)**) and were stored at temperatures below -40°C until analysis. DNA was extracted from 100 - 200 mg of frozen stool using a QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. The concentration and purity of the extracted DNA were assessed by measuring the absorbance at 260 and 280 nm, respectively. Real-time PCR was conducted to quantify the 16S rRNA gene in 6 bacterial species/groups using a SYBR Green detection system. This was performed using the Mx3000P QPCR System (Agilent Technologies Inc., Santa Clara, CA, USA) using a SYBR Premix Ex Taq II (Tli RNase H Plus) Kit (Takara Bio Inc. Kyoto, Japan) and specific primers for the target bacteria. The sequences of the primers (F: forward 5' to 3', R: reverse 5' to 3') are available upon request. The abbreviations for the six target bacteria are as follows: Lactobacilli (Lacto), Bifidobacteria (Bif), Clostridium butyricum (Cbu), Clostridium clostridioforme (Ccl), Clostridium perfringens (Cpe), and Desulfovibrio (Dsv). A universal primer (Uni) set was employed to amplify the conserved regions of the 16S rRNA gene in bacteria and was used as a reference to express the relative quantity of the target bacteria, as detailed below.

The PCR reaction was conducted in a total volume of 10 μ L, comprising 5 μ L of SYBR Premix Ex Taq, 0.2 μ L of ROX Reference Dye, 0.1 μ M of each primer, and 25 ng of template DNA. The PCR conditions included an initial denaturation at 95°C for 10 seconds, followed by 35 cycles of denaturation at 95°C for 5 seconds and annealing/extension at 60°C for 30 seconds. After each amplification, a dissociation curve analysis was performed to confirm PCR specificity. DNA standards for calibration were prepared from serially diluted PCR products that were previously amplified from macaque stool DNA using Uni primers. To quantify the target bacterial DNA in stool samples, the amount of the target DNA was calculated by dividing the quantity of interest by that obtained using Uni for normalization. Amplification and detection were performed in duplicate.

2.10. RNA Isolation

To isolate RNA for DNA microarray analyses, iliac lymph node samples were collected from individual mouse within 3 h on the same day to minimize the influence of circadian clock genes. Each tissue sample was homogenized using RNAiso Plus. Total RNA was extracted as described previously [15] [16]. Only RNA samples of exceptional quality were used for the microarray examination, characterized by an A260/A230 ratio of 1.5, an A260/A280 ratio of 1.8, and RNA integrity number (RIN) value of 6.0.

2.11. DNA Microarray

Thus obtained RNA samples were pooled by three groups, control group (Ctr) and two experimental groups, Polli and SBGN + Polli. DNA microarray analysis was performed on the three groups. Complementary DNA (cDNA) and Cy3-labeled complementary RNA (cRNA) were prepared as described previously [15] [16]. The labeled cRNA was fragmented using the Gene Expression Hybridization Kit and applied to Whole Mouse Genome Array Ver. 2.0 slides (Agilent, 4x 44K). Following hybridization at 65°C for 17 hours, the slides were washed with Gene Expression Wash Buffers 1 and 2 in accordance with the manufacturer's instructions. The slides were subsequently scanned using a GenePix 4000 B scanner (Molecular Devices, San Jose, CA, USA).

The scanned images were converted into a digital format and standardized normalized using GenePix Pro 6.0 software (Molecular Devices).

2.12. Bioinformatic for Microarray Data Analyses

In the experimental group, we identified genes that exhibited > 2-fold upregulation or < 0.5-fold downregulation in expression compared with the control group. These genes were annotated, and databases such as PubMed, NCBI, and Google, and other information sources were used to search for references. We analyzed the immune-related functions of the annotated genes to gain insights into the potential mechanisms underlying the mitigating effects of SBGN, while also utilizing additional relevant information sources for reference.

2.13. Statistical Analysis

All test results are expressed as the mean with standard deviation. Student's or Aspin-Welch t-test was used for comparisons between two groups after the equality of the two variances had been examined by an F-test. All analyses were conducted using StatLight software (Yukms, Co., Ltd., Kanagawa, Japan). A p-value ≤ 0.05 was considered statistically significant.

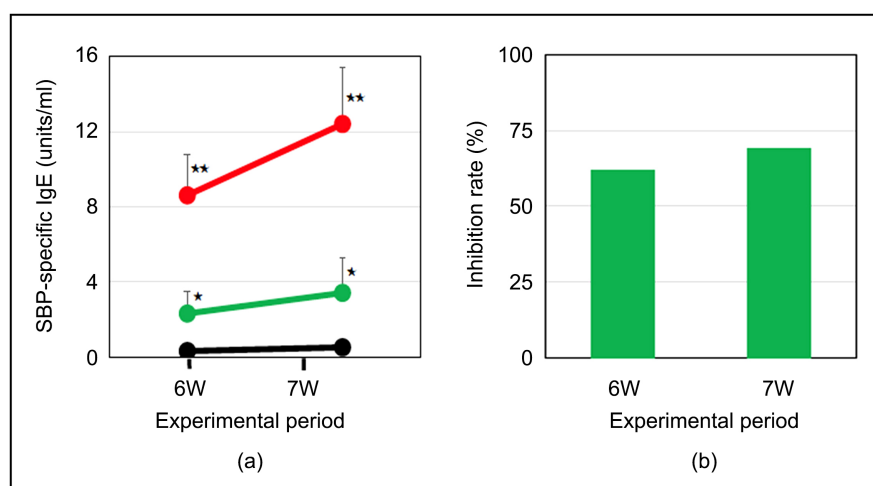
3. Results

In preliminary examinations of the dose-response of SBGN within the range of 3 - 10 mg/kg in mice, we obtained reasonable results with the oral administration

of 10 mg/kg. This study was conducted using an oral dosage of 10 mg/kg of SBGN in mice. Employing the methodologies illustrated in **Figure 1(a)** and **Figure 1(b)**, we acquired the subsequent results.

3.1. Inhibitory Effect of SBGN on Antigen-Specific IgE Production in Pollinosis Mice Model Sensitized with Sugi-Pollen Antigen

Figure 2(a) shows the plasma levels of SBP-specific IgE in pollinosis mice sensitized with sugi-pollen antigen (Polli) compared with those of Ctr and SBGN + Polli. The plasma levels of SBP-specific IgE in Polli were 8.4 at 6 weeks and 12.2 units at 7 weeks, respectively. In contrast, SBP-specific IgE levels in the SBGN + Polli group decreased to 3.2 at 6 weeks and 3.8 units at 7 weeks, individually. This indicated that pre-administration of SBGN reduced SBP-specific IgE production in pollinosis mice (Polli). Thus, oral administration of SBGN inhibited SBP-specific IgE production in pollinated animals by 68% to 72% (**Figure 2(b)**).

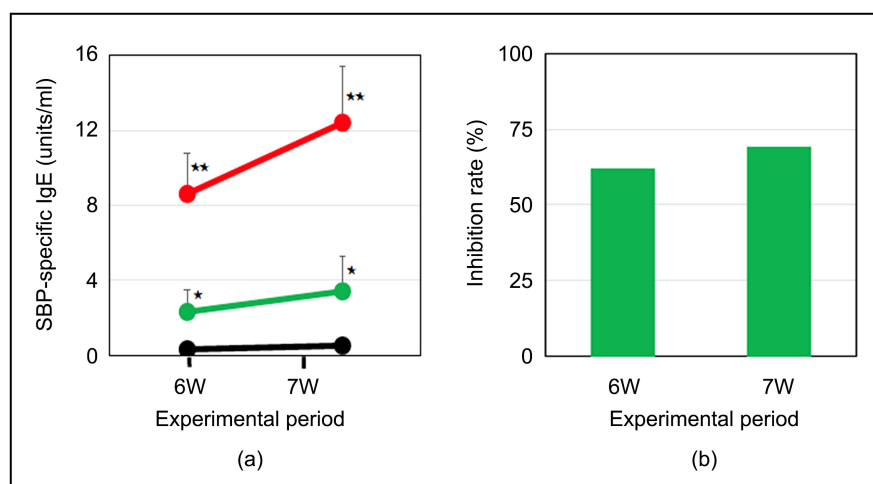


(a) shows plasma level of SBP-specific IgE in three groups, **Ctr (black)**, **Polli (red)**, and **SBGN + Polli (green)**. SBP-specific IgE was measured by ELISA using plasma samples from the three groups at 6 and 7 weeks, respectively. (b) displays SBGN-mediated inhibition to the SBP-specific IgE production in pollinosis mice (Polli) at 6 and 7 weeks, respectively. Inhibition rate (%) was calculated from the plasma level of SBP-specific IgE in Polli and SBGN + Polli. ** $p < 0.01$, Polli compared with Ctr. * $p < 0.05$, SBGNPolli compared with Polli.

Figure 2. Inhibitory effect of SBGN on SBP-specific IgE production in pollinosis mice.

3.2. Inhibitory Effect of SBGN on SBP-Induced Sneezing in the Pollinosis Mice Model

Figure 3(a) displays the number of SBP-induced sneezes among three groups, PBS, Polli, and SBGN + Polli. The numbers of SBP-induced sneezes in Polli were 42 times/5 min at 6 weeks and 47 times/5 min at 7 weeks, respectively. Whereas the sneezing frequencies in the SBGN + Polli group were 14 and 18 times/5 min at 6 and 7 weeks, individually. These results suggest that SBGN administration inhibited SBP-induced sneezing in pollinosis animals by 64% - 72% (**Figure 3(b)**).



(a) shows the number, sneezing time/5 min, of SBP-induced sneezes among three groups, **Ctrl (black)**, **Polli (red)**, and **SBGN + Polli (green)**. (b) displays SBGN-mediated inhibition to the SBP-induced sneezing in pollinosis mice (Polli) at 6 and 7 weeks, respectively. Inhibition rate (%) was calculated from the sneezing time/5 min in Polli and SBGN + Polli. ** $p < 0.01$, Polli compared with Ctrl. * $p < 0.05$, SBGN + Polli compared with Polli.

Figure 3. Inhibitory effect of SBGN on SBP-induced sneezing in the pollinosis mice.

3.3. DNA Microarray Analyses and Bioinformatics

To elucidate the mechanism underlying SBGN-mediated inhibition of SBP-specific IgE production in pollinosis mice model, we performed DNA microarray analyses using RNA extracted from the iliac lymph nodes of three groups, Ctrl, Polli, and SBGN + Polli, at 7 weeks.

3.3.1. Amelioratively Downregulated SBGN-Related Genes

We compared the gene expression profiles of the two groups, Ctrl and Polli, and identified 45 genes that were upregulated by more than 2.0-fold in Polli (data not shown). In the subsequent comparison of the upregulated gene expression profiles between Polli and SBGN + Polli, we aimed to identify genes associated with the immune response, inflammation, and tissue repair that were downregulated in SBGN + Polli. As shown in **Table 1**, we identified seven genes that were downregulated by more than 0.5-fold in the SBGN + Polli and were related to the ameliorative action of SBGN on pollinosis. These seven genes were referred to as amelioratively downregulated SBGN-related genes. **Table 1** lists these amelioratively downregulated SBGN-related genes, including *Mnd1* (meiotic nuclear divisions 1 homolog), *Igk* (immunoglobulin kappa chain), *Igh* (immunoglobulin heavy chain variable region), *Aicda* (activation-induced cytidine deaminase), *Slpr2* (Sphingosine-1-phosphate receptor 2), *U76382.1* (immunoglobulin light chain mRNA), and *EndoU* (evolutionarily conserved single-stranded RNA binding protein, a novel regulator of B-cell activation-induced cell death).

3.3.2. Amelioratively Upregulated SBGN-Related Genes

By comparing the gene expression profiles between the two groups, Ctrl and Polli,

we identified 73 genes that were downregulated by a factor of 0.5 or lower in the Polli group (data not shown). We then analyzed the downregulated gene expression profiles between Polli and SBGN + Polli groups to select genes associated with immune response, inflammation, or tissue repair that were upregulated in the SBGN + Polli group. As shown in **Table 2**, we extracted twelve genes that were upregulated by a factor of 2.0 or greater in the SBGN + Polli. These genes appear to be associated with SBGN-mediated amelioration of pollinosis. These 12 genes are referred to as amelioratively upregulated SBGN-related genes. **Table 2** lists these amelioratively upregulated genes, which include *Ngfr* (Nerve growth factor receptor), *Prg2* (Proteoglycan 2, bone marrow), *Ear6* (Eosinophil-associated, ribonuclease A family, member 6), *Ch3B* (Chitinase 3-like 3), *Pon1* (Paraoxonase 1), *Cd209b* (CD209b antigen, transcript variant 2), *Ear1* (Eosinophil-associated, ribonuclease A family, member 1), *Ms4a8a* (Membrane-spanning 4-domains, subfamily A, member 8A), *Camp* (Cathelicidin antimicrobial peptide), *Ltf* (Lactotransferrin), *Elane* (Elastase, neutrophil expressed), and *Ngp* (Neutrophilic granule protein).

Table 1. Amelioratively downregulated SBGN-related genes.

Gene Symbol	Product: Description [Reference]	Fold change	
		Polli ² /Ctr ¹	SBGN + Polli ³ /Polli ² (SBGN + Polli ³ /Ctr ¹)
<i>Mnd1</i>	MIND1: Meiotic nuclear divisions 1 homolog (<i>S. cerevisiae</i>) [22]	3.6	0.3 (1.2)
<i>Igk</i>	IgK: Immunoglobulin kappa chain [19]-[21]	6.0	0.3 (2.0)
<i>Igh</i>	IgH: Immunoglobulin heavy chain variable region [19]-[21]	6.3	0.3 (2.1)
<i>Aicda</i>	AICDA: Activation-induced cytidine deaminase [23]	25.4	0.4 (10.2)
<i>Slpr2</i>	S1PR2: Sphingosine-1-phosphate receptor 2 [24]	2.8	0.4 (1.1)
<i>U76382.1</i>	Immunoglobulin kappa light chain, Anti-CD4 immunoglobulin light chain mRNA [19]-[21]	2.2	0.5 (1.1)
<i>Endou</i>	ENDOU: Endonuclease, poly(U) specific, Novel regulator of B-cell activation-induced cell death [25]	7.6	0.5 (3.8)

¹Ctr: Control group; ²Polli: Sugi pollen sensitized group; ³SBGN + Polli: SBGN pre-administrated Polli group.

Table 2. Amelioratively upregulated SBGN-related genes.

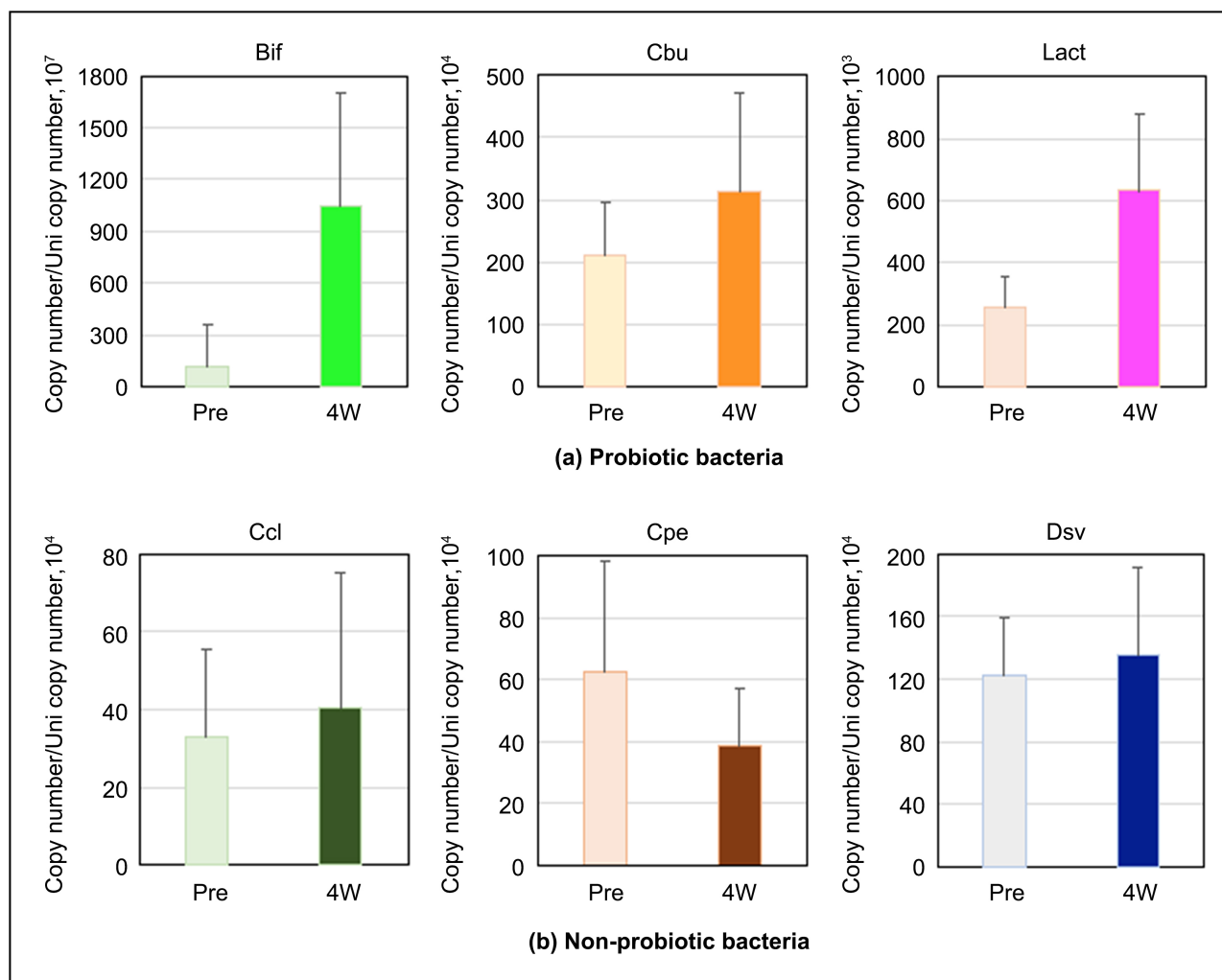
Gene Symbol	Product: Description [Reference]	Fold Change	
		Polli ² /Ctr ¹	SBGN + Polli ³ /Polli ² (SBGN + Polli ³ /Ctr ¹)
<i>Ngfr</i>	NGFR: Nerve growth factor receptor (TNFR superfamily, member 16) [26]	0.1	3.0 (0.3)
<i>Prg2/Mbp</i>	PRG2/MBP: Proteoglycan 2 bone marrow, also named eosinophil major basic protein (MBP) [27]	0.1	3.0 (0.3)
<i>Ear6</i>	EAR6: Eosinophil-associated, ribonuclease A family, member 6 [28]	0.1	3.0 (0.3)
<i>Chi3l3/Ym1</i>	CHI3L3/YM1: Chitinase 3-like 3, also named Ym1 [29]-[31]	0.2	2.5 (0.5)
<i>Pon1</i>	PON1: Paraoxonase 1 [32]	0.2	3.0 (0.6)
<i>Cd209b</i>	CD209b antigen, transcript variant 2 [33]	0.2	2.5 (0.5)
<i>Ear1</i>	EAR1: Eosinophil-associated, ribonuclease A family, member 1 [34]	0.2	2.5 (0.5)
<i>Ms4a8a</i>	MS4A8A: Membrane-spanning 4-domains, subfamily A, member 8A [35]	0.3	2.3 (0.7)
<i>Camp</i>	CAMP: Cathelicidin antimicrobial peptide [36]	0.3	2.3 (0.7)
<i>Ltf</i>	LTF: Lactotransferrin [37]	0.4	2.0 (0.8)
<i>Elane</i>	ELANE/NE: Elastase, neutrophil expressed [38]	0.4	2.0 (0.8)
<i>Ngp</i>	NGP: Neutrophilic granule protein [41]	0.5	2.0 (1.0)

¹Ctr: Control group; ²Polli: Sugi pollen sensitized group; ³SBGN + Polli: SBGN pre-administrated Polli group.

3.4. Effect of SBGN on Gut Microbiota

We also examined the effect of oral SBGN administration on the gut microbiota of mice in a separate study, as outlined in **Figure 1(b)**. Because of significant individual differences in the gut microbiota, conducting comparative studies on changes between the control and SBGN-administered groups, which consisted of

a limited number of animals (*i.e.*, 6 - 8 mice), was challenging. Therefore, in this study, we utilized stool samples collected from the same animals before (Pre) and after (4W) SBGN administration to compare the effects of SBGN on gut microbiota at these two time points, Pre and 4W. We performed real-time PCR using DNA extracted from stool samples to assess the levels of six target bacteria, which were probiotic bacteria including Bifidobacteria (Bif); *Clostridium butyricum* (Cbu); and Lactobacilli (Lact) and non-probiotic bacteria including *C. clostridiiforme* (Ccl); *C. perfringens* (Cpe); and *Desulfovibrio* (Dsv).



(a) shows the levels of three probiotic bacteria, Bifidobacteria (Bif); *Clostridium butyricum* (Cbu); and Lactobacilli (Lact), after four weeks of SBGN administration. (b) displays the levels of three non-probiotic bacteria, *C. clostridiiforme* (Ccl); *C. perfringens* (Cpe); and *Desulfovibrio* (Dsv), during four weeks of SBGN administration. Bars at Pre and 4W mean SEM.

Figure 4. Effect of SBGN on gut microbiota.

As shown in **Figure 4(a)**, the levels of three probiotic bacteria, Bif, Cbu, and Lact, increased 8.7-, 1.5-, and 2.4 -fold, respectively, after four weeks of SBGN administration. Conversely, the levels of three non-probiotic bacteria, Ccl, Dsv, and Cpe, barely changed or decreased by 0.4-fold during four weeks of SBGN ad-

ministration (**Figure 4(b)**). Very little alteration in the levels of both probiotic and non-probiotic bacteria was noted in fecal samples from control mice following 4 weeks of PBS administration. (data not shown).

These results indicated that SBGN administration led to an increase in probiotic bacteria, particularly Bif, in the gut microbiota.

4. Discussions

4.1. Sugi-Pollinosis, IBGNs, and SBGNs

Sugi-pollinosis is caused by the SBP-specific IgE antibodies, which bind to FcεRI, a high-affinity receptor that is expressed on the surface of mast cells and/or basophils. The initial step in the development of pollinosis includes production of SBP-specific IgE antibodies. Therefore, certain foods and related materials that suppress the production of SBP-specific IgE may help alleviate sugi-pollinosis.

There are two types of β -glucans: IBGNs and SBGNs. IBGNs activate dectin-1 signaling in various immune-inflammatory cells, including B and T cells, antigen-presenting cells, mast cells, and granulocytes (neutrophils and eosinophils). Activation of these cells by IBGNs begins with their binding to dectin-1, which is present on the cell surface. This triggers oligomerization and activates the Src-Syk signaling cascade, leading to an immune-inflammatory response [1]-[4]. In contrast, SBGNs acts as a dectin-1 antagonist; it binds to dectin-1 but does not initiate any intracellular signaling pathway [1]. Consequently, SBGNs inhibit the action of IBGNs by competing for binding to dectin-1 and preventing IBGN-mediated activation of the intracellular machinery [12] [13].

During the immune response, IBGNs function as adjuvants leading to the production of specific antibodies, including IgE. Pollen grains contain allergy-related components, allergens, and IBGNs, similar to adjuvants, which initiate allergic immune responses [8]. Japanese cedar pollen contains IBGN polysaccharides and exhibits IgE-inducing activity in mice [9]. Kanno *et al.* reported that sugi pollen contains IBGNs that stimulate dectin-1 signaling and demonstrate a potent adjuvant effect under allergic conditions, promoting Th2 imbalance and SBP-specific IgE production [11]. In the present study, we leveraged the adjuvant effect of IBGNs to successfully establish a sugi pollinosis mouse model through sensitization with only sugi pollen. This was done without the use of other adjuvants such as alum, which is commonly used to create allergy models associated with specific IgE production.

Previous studies have reported that SBGNs exhibit anti-allergic effects [12] [13], including in humans [14]. We confirmed the anti-allergic actions of SBGNs by demonstrating the inhibition of both SBP-specific IgE production and SBP-induced sneezing by oral pre-administration in a sugi-pollinosis mouse model (**Figure 2** and **Figure 3**). The anti-allergic effect mediated by SBGNs likely results from their competition with adjuvant IBGNs for binding to dectin-1, which subsequently inhibits intracellular mechanisms involved in allergic immune responses. However, detailed information regarding the molecular mechanisms underlying

the anti-allergic action of orally administered SBGNs has not yet been adequately gathered.

4.2. DNA Microarray Analyses and Iliac Lymph Nodes

An efficient method for studying the expression levels of most genes in an organism's functional genome, particularly the transcriptional product (mRNA), is through DNA microarray techniques, which provide high-throughput analysis of transcripts/mRNAs. In our previous studies on sublingual vaccination [15] [16], we elucidated novel molecular events within the immune system and their related responses using a DNA microarray approach. In the present study, we further studied the mechanism underlying the anti-allergic action of orally administered SBGNs in a sugi-pollen allergy model. DNA microarray analyses were conducted using RNA extracted from the iliac lymph nodes, as these nodes are local lymphatic tissues located near the tail base muscle, where the sugi-pollen antigen was intramuscularly injected to sensitize the animals to pollinosis. A highly effective immune response, known as sensitization, has been reported to occur in lymphocytes from the iliac lymph nodes following the intramuscular injection of a sensitizing antigen at the tail base in mice [17]. Therefore, a method involving the iliac lymph nodes, through intramuscular sensitization at the tail base and subsequent collection of these lymph nodes, was established to produce specific antibodies [18]-[21]. This method has also been utilized in studies examining allergies and related immune-inflammatory responses.

4.3. Amelioratively Downregulated SBGN-Related Genes

Using DNA microarray analysis, we identified four additional SBGN-related genes that were downregulated, including *Mnd1* (meiotic nuclear division 1 homolog), *Aicda* (activation-induced cytidine deaminase), *S1pr2* (Sphingosine-1-phosphate receptor 2), and *EndoU* (evolutionarily conserved single-stranded RNA-binding protein, a novel regulator of B-cell activation-induced cell death) (Table 1). MND1 is involved in DNA repair during cell growth and is related to the cell cycle, proliferation, invasion, and migration of cells within the lymph node microenvironment [22]. Consequently, MND1 appears to be associated with the stimulation of the immune response through the infiltration of immune cells into the lymph nodes. Reportedly, AICDA exhibits the highest expression in the spleen of OVA-sensitized allergic mice [23], with its gene expression significantly promoted by IL-21 and IL-17A [23]. S1PR2 is a receptor for sphingosine-1-phosphate (SIP), an important signaling molecule that is associated with allergic inflammatory diseases, including pollen allergies, asthma, and anaphylaxis [24]. The interaction between S1PR2 and SIP on mast cells enhances their activation, contributing to allergic inflammation [24]. ENDOU serves as a critical regulator of the pathway that controls B cell survival and antigen responsiveness, contributing to B cell tolerance [25]. ENDOU is associated with the survival of pathogenic B cells, such as those responding to sugi pollen antigens in sugi pollen allergies. As these four genes

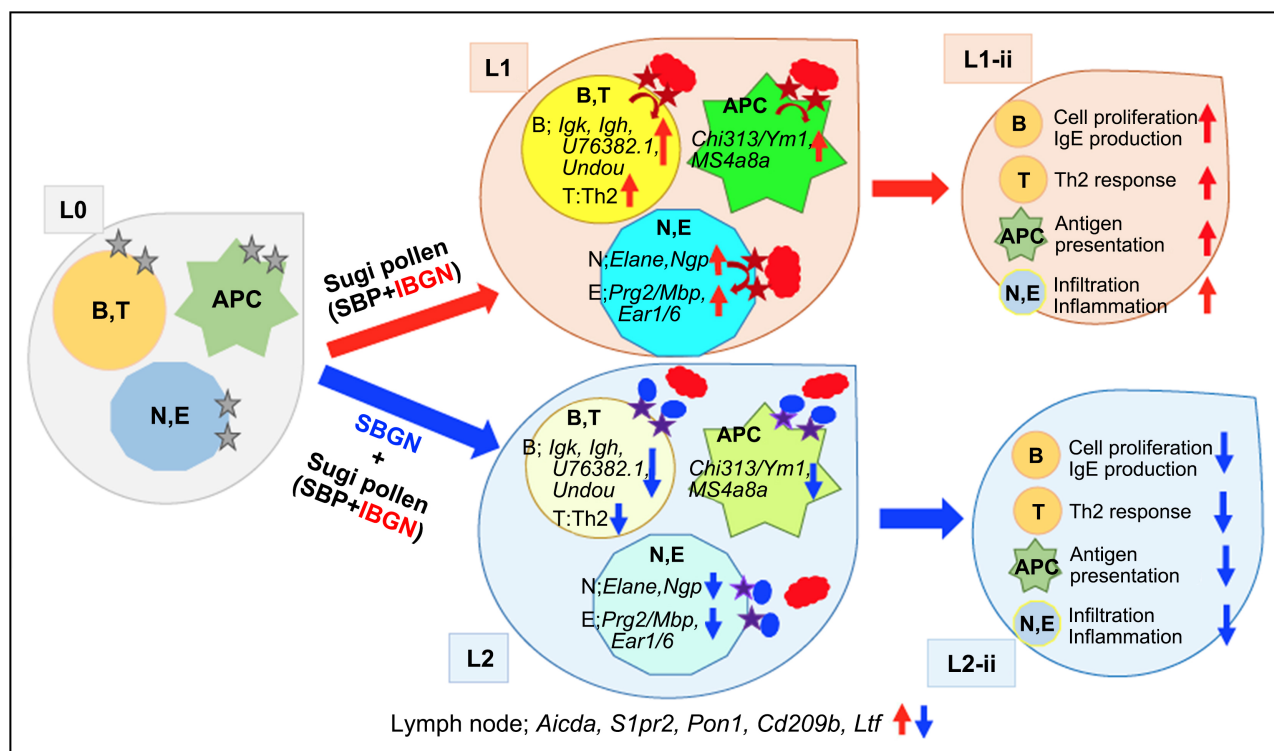
directly or indirectly participate in the dysregulated stimulation of the immune system, the upregulation of these genes in Polli mice correlates with an allergic state in local lymphoid tissues, such as the iliac lymph nodes. Conversely, the downregulation of these upregulated genes in SBGN-administered mice (SBGN + Polli) ameliorated the dysregulated immune response, including allergic reactions. Therefore, SBGN administration appeared to ameliorate sugi pollen allergies.

4.4. Amelioratively Upregulated SBGN-Related Genes

As revealed by the ameliorative upregulation of SBGN-related gene (**Table 2**), we uncovered 12 genes whose expressions were upregulated in SBG-administrated mice, SBGN + Polli, comparison with those in sugi pollen allergy mice, Polli. NGFR is a nerve growth factor (NGF) receptor that dampens the inflammatory response and limits tissue damage through the NGFR-NGF interaction [26]. Thus, NGFR likely contributes to regulatory feedback on the development and maintenance of chronic inflammation, including allergies. PRG2, also known as eosinophil major basic protein (MBP), is involved in the antiparasitic defense system as a helminth toxin and in immune hypersensitivity reactions. MBP also participates in tissue remodeling in allergic atopy through its pro-angiogenic effects [27]. EAR6 belongs to the eosinophil-derived RNase A superfamily and is involved in innate and Ag-specific immune responses to danger signals such as endogenous multifunctional immune alarmins [28]. CHI3L3/YM1 is primarily produced by macrophages and has multifunction [29]. CHI3L3/YM1 stimulates the Th2 response in the early phase of allergic inflammation [30], but also inhibits the Th2 response by reducing the gene expression of Th2 cytokines (IL-5 and IL-13), resulting in the regulation of Th2 imbalance [31]. Thus, upregulation of CHI3L3/YM1 in SBGN + Polli is thought to relate to anti-allergic effect of SBG administration. PON1 is a major antioxidant enzyme and acts to ameliorate Th2-mediated inflammation by down-regulating IL-4 and IL-13 and upregulating TGF- β [32]. EAR1 associates with anti-inflammatory reaction to regulate macrophage and polarization and block NF- κ B activation [34]. MS4A8A is a CD20 homolog expressed in M2 macrophages and plays a role in integrating anti-inflammatory signals into M2-like macrophages [35]. CAMP is an immunomodulatory peptide mainly secreted by immune cells, macrophages and lymphocytes, and has a regulatory role of inflammatory mediators, such as TNF- α [36]. LTF has multiple physiological functions, including anti-inflammatory effects, and exhibits protective effects by reducing the expression of Th2 cytokines and secretion of allergen-specific antibodies in an OVA-induced allergic mouse model [37]. ELANE/NE plays a role in dampening inflammatory reactions by degrading various pro-inflammatory cytokines, including IL-1, TNF, and IL-6 [38]. Neutrophils are known to participate in both the initiation and resolution of inflammation [39] [40], and neutrophil granule proteins (NGP) play a role in minimizing inflammatory damage to neighboring tissues through modulation of neutrophil trafficking, migration, and/or extracellular trap formation [41]. Following pollen antigen sensitization in Polli mice, the

iliac lymph nodes were enlarged due to inflammatory events accompanied by the infiltration of immune-inflammatory cells, macrophages, lymphocytes (B and T cells), and granulocytes (neutrophils, basophils, and eosinophils) (data not shown). In turn, SBGN administration to SBGN + Polli mice relieved iliac lymph node enlargement. As mentioned above, these 12 upregulated genes were expressed in immune-inflammatory cells within the iliac lymph nodes, and SBGN administration dampened the inflammatory events by upregulating the expression of these genes in the lymph nodes.

4.5. Mechanism Underlying Anti-Allergy Action Mediated by SBGN on Sugi Pollinosis



L0, L1/LI-ii, and L2/L2-ii indicate steady state, allergic condition generated by sugi pollen (SBP + IBGN), and mitigated status caused by SBGN, respectively, in iliac lymph nodes. B, T, APC, N, and E mean B- and T-cells, antigen-presenting cells, neutrophils, and eosinophils, respectively. **Gray, brown, and purple** stars represent the dectin-1 receptor expressed on the aforementioned cells, separately. **Red solid clouds** in both L1 and L2, along with a **blue solid circle** in L2, indicate **insoluble β -glucan (IBGN)** and **soluble β -glucan (SBGN)**, individually. In L1, genes with **red upward arrows** indicate sugi pollen (SBP + IBGN)-induced upregulated expression in the above-mentioned immune-inflammatory cells. In L2, genes with **blue downward arrows** mean amelioratively downregulated SBGN-related genes in these cells. In L1-ii, immune-inflammatory reactions with **red upward arrows** indicate sugi pollen (SBP + IBGN)-induced activation status in each cell. On the other hand, in L2-ii, these reactions with **blue downward arrows** represent SBGN-mediated alleviating states in the immune-inflammatory cells of iliac lymph nodes.

Figure 5. Possible mechanism underlying anti-allergy action mediated by soluble β -glucan (SBGN) on Japanese cedar pollinosis.

Figure 5 illustrates possible molecular events occurring in the aforementioned immune-inflammatory cells of iliac lymph nodes, which are in an allergic state (L1 and L1-ii) induced by sugi pollen (SBP + IBGN) or an ameliorated allergic

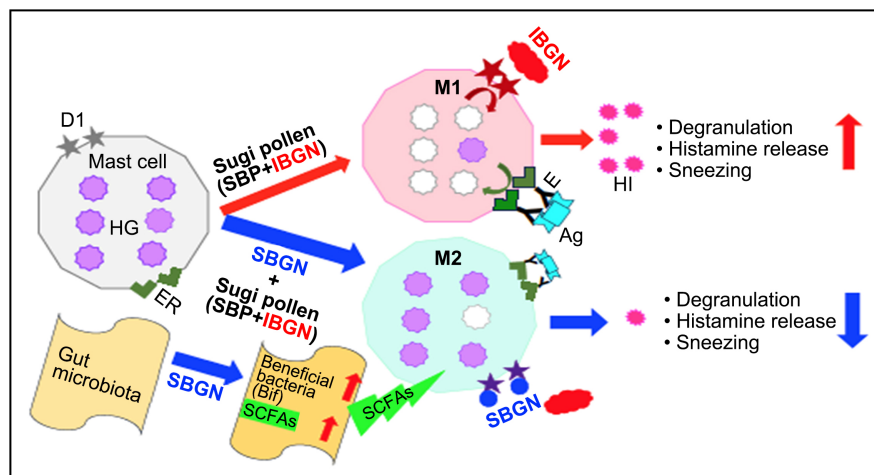
condition (L2 and L2-ii) resulting from SBGN. **Figure 5** suggests that SBGN inhibits the binding of IBGN to dectin-1 on several immune-inflammatory cells, together with the modulation of upregulated expression of SBGN-related genes linked to the IBGN-mediated allergic responses in the lymph nodes (L1 and L2). This figure also implies the alleviation of IBGN-stimulated immune-inflammatory reactions in the related cells (LI-ii and L2-ii).

4.6. Suppression of Mast Cell Activation by SBGN

Mast cells play a pivotal role in initiating and sustaining inflammation, particularly in allergic reactions. Upon re-exposure to allergens, IgE-mediated aggregation of the IgE receptor, FcεRI, occurs on the mast cell surface, leading to rapid degranulation of the mast cells [42]. Antigen-induced sneezing is a common allergic symptom. This occurs when intranasal mast cells are activated by the binding of antigens to the antigen-specific IgE and FcεRI complex, which is followed by degranulation and the release of inflammatory mediators, including histamine. SBGN directly inhibits antigen-induced mast cell activation, resulting in anti-allergic effects [14] [43]. In the current study, we also found that oral administration of SBGN inhibited SBP-induced sneezing in SBG + Polli mice (**Figure 3(a)** and **Figure 3(b)**). This inhibition is believed to stem from the suppressive effect of SBGN on mast cell activation via dectin-1. Mast cells are known to express dectin-1, and a dectin-1 antagonist, SBGN (laminarin), has been shown to block the signaling pathways that lead to mast cell activation through dectin-1 signaling [44].

Another mechanism contributing to the SBGN-mediated inhibition of mast cell activation has also been reported. In this instance, antigen-mediated mast cell activation is suppressed by short-chain fatty acids (SCFAs), which are metabolic products of the gut microbiota [45]. A previous study indicated that oral administration of yeast-derived SBGN acted as a prebiotic, altering the gut microbiota to increase the abundance of probiotic bacteria, resulting in elevated levels of fecal SCFAs, including acetic, propionic, and butyric acids [46]. In this study, we also found that SBGN administration over a 4-week period resulted in changes in the gut microbiota profile and an increase in probiotic bacteria, specifically Bif (**Figure 4**). Additionally, oral administration of β -glucan caused an increase in probiotic bacteria, accompanied by higher levels of SCFAs, including butyric acid, lactic acid, and propionic acid [47] [48]. Although we did not obtain direct evidence for SBGN-mediated accumulation of SCFAs in this study, it is plausible that SBGN functions as a prebiotic, suppressing mast cell activation through SCFAs produced by gut microbiota, which may help mitigate sugi pollinosis and other related allergies.

Figure 6 presents a possible mechanism by which SBGN inhibits mast cell activation. Briefly, SBGN appears to compete with IBGN for binding to dectin-1, reduces FcεRI stimulation due to lower levels of SBP-specific IgE, and is modulated by SCFA regulation. These factors contribute to the suppression of mast cell degranulation, leading to inhibit histamine release and reduced sneezing.



M1 and M2 mean mast cells of an activated/degranulated state mediated by sugi pollen (M1) and an ameliorated status (M2) induced by SBGN, respectively. **D1**, **HG**, **ER**, **SCFAs**, **IBGN**, **E**, **Ag**, **SBGN**, and **HI** mean **dectin-1receptor**, **histamine granule**, **FcεRI**, short-chain fatty acids, **insoluble β-glucan particles**, **SBP-specific IgE**, **SBP antigen**, **soluble β-glucan**, and **histamine**, individually.

Figure 6. Possible mechanism for SBGN-mediated inhibitory action on mast cell activation.

5. Conclusions

IBGN acted as a dectin-1 agonist and was present in sugi pollen as a polysaccharide adjuvant that enhanced the immune-inflammatory response. This response led to the production of SBP-specific IgE and the activation of mast cells, resulting in sneezing associated with SBP. In contrast, SBGN, a dectin-1 antagonist derived from black yeast, mitigated sugi pollen allergy by reducing SBP-specific IgE production and decreasing SBP-induced sneezing in a mouse model of sugi pollinosis. The anti-allergic effects of SBGN were mediated by preventing the IBGN-stimulated immune-inflammatory response in, possibly, B and T cells, antigen-presenting cells, and granulocytes.

Additionally, it appeared that SBGN inhibited mast cell activation by reducing SBP-specific IgE levels and increasing gut probiotic bacteria, which subsequently leads to the production of short-chain fatty acids (SCFAs). Therefore, SBGN and related functional foods and/or beverages appear to be advantageous for controlling allergies.

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Author Contributions

Conceptualization, M.T., T.S. and S.N.; Investigation, F.M. and S.N.; Methodol-

ogy, F. M.; Interpretation of data and Project administration, M.T. and K.M.; Supervision, M.T. and T.S.; Validation, K.M.; Writing - original draft, M.T. and S. N.

Institutional Review Board Statement

This study was conducted according to the guidelines of Institutional Animal Care and Committee Guide of Intelligence and Technology Lab, Inc. (ITL) based on the Guidelines for Proper Conduct of Animal Experiments and approved by the Animal Care Committee of the ITL (approved number: AE2023011 date: 11 September 2023). This study was also approved by the ITL Biosafety Committee (approved number: BS2023011, date: 11 September 2023).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] De Marco Castro, E., Calder, P.C. and Roche, H.M. (2021) Back Cover: B-1,3/1,6-Glucans and Immunity: State of the Art and Future Directions. *Molecular Nutrition & Food Research*, **65**, e1901071. <https://doi.org/10.1002/mnfr.202170003>
- [2] Novak, M. and Vetvicka, V. (2008) β -Glucans, History, and the Present: Immunomodulatory Aspects and Mechanisms of Action. *Journal of Immunotoxicology*, **5**, 47-57. <https://doi.org/10.1080/15476910802019045>
- [3] Goodridge, H.S., Reyes, C.N., Becker, C.A., Katsumoto, T.R., Ma, J., Wolf, A.J., *et al.* (2011) Activation of the Innate Immune Receptor Dectin-1 upon Formation of a 'Phagocytic Synapse'. *Nature*, **472**, 471-475. <https://doi.org/10.1038/nature10071>
- [4] Dulal, H.P., Adachi, Y., Ohno, N. and Yamaguchi, Y. (2018) β -Glucan-Induced Cooperative Oligomerization of Dectin-1 C-Type Lectin-Like Domain. *Glycobiology*, **28**, 612-623. <https://doi.org/10.1093/glycob/cwy039>
- [5] Allakhverdi, Z., Bouguermouh, S., Rubio, M. and Delespesse, G. (2005) Adjuvant Activity of Pollen Grains. *Allergy*, **60**, 1157-1164. <https://doi.org/10.1111/j.1398-9995.2005.00861.x>
- [6] Chen, X. and Kim, J. (2009) Callose Synthesis in Higher Plants. *Plant Signaling & Behavior*, **4**, 489-492. <https://doi.org/10.4161/psb.4.6.8359>
- [7] Zavaliev, R., Ueki, S., Epel, B.L. and Citovsky, V. (2010) Biology of Callose (β -1,3-Glucan) Turnover at Plasmodesmata. *Protoplasma*, **248**, 117-130. <https://doi.org/10.1007/s00709-010-0247-0>
- [8] Kanno, T., Kim, C., Yamanaka, D., Ishibashi, K., Tanaka, H., Ohno, N., *et al.* (2021) Possibility of Japanese Cedar Pollen Causing False Positives in the Deep Mycosis Test. *International Journal of Molecular Sciences*, **22**, Article 2135. <https://doi.org/10.3390/ijms22042135>
- [9] Kamijo, S., Takai, T., Kuhara, T., Tokura, T., Ushio, H., Ota, M., *et al.* (2009) Cupressaceae Pollen Grains Modulate Dendritic Cell Response and Exhibit Ige-Inducing Adjuvant Activity *in Vivo*. *The Journal of Immunology*, **183**, 6087-6094. <https://doi.org/10.4049/jimmunol.0901039>
- [10] Angelina, A., Martín-Cruz, L., de la Rocha-Muñoz, A., Lavín-Plaza, B. and Palomares, O. (2023) C-Type Lectin Receptor Mediated Modulation of T2 Immune Re-

- sponses to Allergens. *Current Allergy and Asthma Reports*, **23**, 141-151. <https://doi.org/10.1007/s11882-023-01067-0>
- [11] Kanno, T., Adachi, Y., Ohashi-Doi, K., Matsuhara, H., Hiratsuka, R., Ishibashi, K., *et al.* (2021) Latent 1,3-B-D-Glucan Acts as an Adjuvant for Allergen-Specific Ige Production Induced by Japanese Cedar Pollen Exposure. *Allergology International*, **70**, 105-113. <https://doi.org/10.1016/j.alit.2020.08.004>
 - [12] Suzuki, T., Kusano, K., Kondo, N., Nishikawa, K., Kuge, T. and Ohno, N. (2021) Biological Activity of High-Purity B-1,3-1,6-Glucan Derived from the Black Yeast *Aureobasidium Pullulans*: A Literature Review. *Nutrients*, **13**, Article 242. <https://doi.org/10.3390/nu13010242>
 - [13] No, H., Kim, J., Seo, C., Lee, D.E., Kim, J.H., Kuge, T., *et al.* (2021) Anti-Inflammatory Effects of β -1,3-1,6-Glucan Derived from Black Yeast *Aureobasidium Pullulans* in RAW264.7 Cells. *International Journal of Biological Macromolecules*, **193**, 592-600. <https://doi.org/10.1016/j.ijbiomac.2021.10.065>
 - [14] Jippo, T., Suzuki, T., Sato, H., Kobayashi, Y. and Shigekawa, M. (2015) Water-Soluble Low-Molecular-Weight β -(1, 3-1, 6) D-Glucan Inhibit Cedar Pollinosis. *Functional Foods in Health and Disease*, **5**, 80-88. <https://doi.org/10.31989/ffhd.v5i2.173>
 - [15] Yamamoto, T., Mitsunaga, F., Wasaki, K., Kotani, A., Tajima, K., Tanji, M., *et al.* (2023) Mechanism Underlying the Immune Responses of a Sublingual Vaccine for SARS-CoV-2 with RBD Antigen and Adjuvant, Poly(I:C) or AddaS03, in Non-Human Primates. *Archives of Microbiology & Immunology*, **7**, 150-164. <https://doi.org/10.26502/ami.936500113>
 - [16] Yamamoto, T., Hirano, M., Mitsunaga, F., Wasaki, K., Kotani, A., Tajima, K., *et al.* (2024) Molecular Events in Immune Responses to Sublingual Influenza Vaccine with Hemagglutinin Antigen and Poly(I:C) Adjuvant in Nonhuman Primates, *Cynomolgus* Macaques. *Vaccines*, **12**, Article 643. <https://doi.org/10.3390/vaccines12060643>
 - [17] Sado, Y., Inoue, S., Tomono, Y. and Omori, H. (2006) Lymphocytes from Enlarged Iliac Lymph Nodes as Fusion Partners for the Production of Monoclonal Antibodies after a Single Tail Base Immunization Attempt. *Acta Histochemica et Cytochemica*, **39**, 89-94. <https://doi.org/10.1267/ahc.06001>
 - [18] Kobayashi, T., Namba, M., Kohno, M., Koyano, T., Sado, Y. and Matsuyama, M. (2022) An Improved Iliac Lymph Node Method for Production of Monoclonal Antibodies. *Development, Growth & Differentiation*, **64**, 38-47. <https://doi.org/10.1111/dgd.12766>
 - [19] Inlay, M.A., Lin, T., Gao, H.H. and Xu, Y. (2006) Critical Roles of the Immunoglobulin Intronic Enhancers in Maintaining the Sequential Rearrangement of *IgH* and *IgK* Loci. *The Journal of Experimental Medicine*, **203**, 1721-1732. <https://doi.org/10.1084/jem.20052310>
 - [20] Kurosaki, T., Shinohara, H. and Baba, Y. (2010) B Cell Signaling and Fate Decision. *Annual Review of Immunology*, **28**, 21-55. <https://doi.org/10.1146/annurev.immunol.021908.132541>
 - [21] Rogosch, T., Kerzel, S., Hoi, K.H., Zhang, Z., Maier, R.F., Ippolito, G.C., *et al.* (2012) Immunoglobulin Analysis Tool: A Novel Tool for the Analysis of Human and Mouse Heavy and Light Chain Transcripts. *Frontiers in Immunology*, **3**, Article 176. <https://doi.org/10.3389/fimmu.2012.00176>
 - [22] Fang, J., Zhen, J., Gong, Y., Ke, Y., Fu, B., Jiang, Y., *et al.* (2022) MND1 Functions as a Potential Prognostic Biomarker Associated with Cell Cycle and Immune Infiltration in Kidney Renal Clear Cell Carcinoma. *Aging*, **14**, 7416-7442. <https://doi.org/10.18632/aging.204280>

- [23] Li, Y., Wang, Y., Sun, N., Yang, H., Zhang, Q., Zhang, X., *et al.* (2019) Activation-Induced Cytidine Deaminase Plays Crucial Role in Ovalbumin-Induced Food Allergy and Promoted by IL-21. *Molecular Immunology*, **114**, 369-377. <https://doi.org/10.1016/j.molimm.2019.08.012>
- [24] Jo, H., Shim, K. and Jeoung, D. (2022) The Crosstalk between FcεRI and Sphingosine Signaling in Allergic Inflammation. *International Journal of Molecular Sciences*, **23**, Article 13892. <https://doi.org/10.3390/ijms232213892>
- [25] Poe, J.C., Kountikov, E.I., Lykken, J.M., Natarajan, A., Marchuk, D.A. and Tedder, T.F. (2013) Endou Is a Novel Regulator of AICD during Peripheral B Cell Selection. *Journal of Experimental Medicine*, **211**, 57-69. <https://doi.org/10.1084/jem.20130648>
- [26] Minnone, G., De Benedetti, F. and Bracci-Laudiero, L. (2017) NGF and Its Receptors in the Regulation of Inflammatory Response. *International Journal of Molecular Sciences*, **18**, Article 1028. <https://doi.org/10.3390/ijms18051028>
- [27] Puxeddu, I., Berkman, N., Nissim Ben Efraim, A.H., Davies, D.E., Ribatti, D., Gleich, G.J., *et al.* (2009) The Role of Eosinophil Major Basic Protein in Angiogenesis. *Allergy*, **64**, 368-374. <https://doi.org/10.1111/j.1398-9995.2008.01822.x>
- [28] NCBI (n.d.) Ear6 Eosinophil-Associated, Ribonuclease A Family, Member 6 [Mus Musculus (House Mouse)]. <https://www.mousephenotype.org/data/genes/MGI:1890463>
- [29] Kang, Q., Li, L., Pang, Y., Zhu, W. and Meng, L. (2022) An Update on Ym1 and Its Immunoregulatory Role in Diseases. *Frontiers in Immunology*, **13**, Article 891220. <https://doi.org/10.3389/fimmu.2022.891220>
- [30] Ikeda, N., Asano, K., Kikuchi, K., Uchida, Y., Ikegami, H., Takagi, R., *et al.* (2018) Emergence of Immunoregulatory Ym1⁺Ly6c^{hi} Monocytes during Recovery Phase of Tissue Injury. *Science Immunology*, **3**, eaat0207. <https://doi.org/10.1126/sciimmunol.aat0207>
- [31] Sutherland, T.E., Rückerl, D., Logan, N., Duncan, S., Wynn, T.A. and Allen, J.E. (2018) Ym1 Induces RELMα and Rescues IL-4Rα Deficiency in Lung Repair during Nematode Infection. *PLOS Pathogens*, **14**, e1007423. <https://doi.org/10.1371/journal.ppat.1007423>
- [32] Jung, J.H., Kang, S.A., Park, J., Kim, S., Yu, H.S., Mun, S.J., *et al.* (2024) Paraoxonase-1 Is a Pivotal Regulator Responsible for Suppressing Allergic Airway Inflammation through Adipose Stem Cell-Derived Extracellular Vesicles. *International Journal of Molecular Sciences*, **25**, Article 12756. <https://doi.org/10.3390/ijms252312756>
- [33] NCBI (n.d.) Cd209b CD209b Antigen [Mus Musculus (House Mouse)]. <https://www.ncbi.nlm.nih.gov/gene/69165>
- [34] Liu, C., Liu, X., Zhang, Y., Liu, J., Yang, C., Luo, S., *et al.* (2021) Eosinophils Protect Mice from Angiotensin-II Perfusion-Induced Abdominal Aortic Aneurysm. *Circulation Research*, **128**, 188-202. <https://doi.org/10.1161/circresaha.120.318182>
- [35] Schmieder, A., Schledzewski, K., Michel, J., Schönhäuser, K., Morias, Y., Bosschaerts, T., *et al.* (2012) The CD20 Homolog Ms4a8a Integrates Pro- and Anti-Inflammatory Signals in Novel M2-Like Macrophages and Is Expressed in Parasite Infection. *European Journal of Immunology*, **42**, 2971-2982. <https://doi.org/10.1002/eji.201142331>
- [36] Höpfinger, A., Schmid, A., Schweitzer, L., Patz, M., Weber, A., Schäffler, A., *et al.* (2023) Regulation of Cathelicidin Antimicrobial Peptide (CAMP) Gene Expression by TNFα and cfDNA in Adipocytes. *International Journal of Molecular Sciences*, **24**, Article 15820. <https://doi.org/10.3390/ijms242115820>
- [37] Lin, C., Chuang, K., Chen, S., Chao, Y., Yen, C., Yang, S., *et al.* (2022) Lactoferrin

- Ameliorates Ovalbumin-Induced Asthma in Mice through Reducing Dendritic-Cell-Derived Th2 Cell Responses. *International Journal of Molecular Sciences*, **23**, Article 14185. <https://doi.org/10.3390/ijms232214185>
- [38] Weng, Q., Zhu, C., Zheng, K., Wu, Y., Dong, L., Wu, Y., *et al.* (2020) Early Recruited Neutrophils Promote Asthmatic Inflammation Exacerbation by Release of Neutrophil Elastase. *Cellular Immunology*, **352**, Article ID: 104101. <https://doi.org/10.1016/j.cellimm.2020.104101>
- [39] Mantovani, A., Cassatella, M.A., Costantini, C. and Jaillon, S. (2011) Neutrophils in the Activation and Regulation of Innate and Adaptive Immunity. *Nature Reviews Immunology*, **11**, 519-531. <https://doi.org/10.1038/nri3024>
- [40] Jones, H.R., Robb, C.T., Perretti, M. and Rossi, A.G. (2016) The Role of Neutrophils in Inflammation Resolution. *Seminars in Immunology*, **28**, 137-145. <https://doi.org/10.1016/j.smim.2016.03.007>
- [41] Othman, A., Sekheri, M. and Filep, J.G. (2021) Roles of Neutrophil Granule Proteins in Orchestrating Inflammation and Immunity. *The FEBS Journal*, **289**, 3932-3953. <https://doi.org/10.1111/febs.15803>
- [42] Gilfillan, A.M. and Tkaczky, C. (2006) Integrated Signalling Pathways for Mast-Cell Activation. *Nature Reviews Immunology*, **6**, 218-230. <https://doi.org/10.1038/nri1782>
- [43] Sato, H., Kobayashi, Y., Hattori, A., Suzuki, T., Shigekawa, M. and Jippo, T. (2012) Inhibitory Effects of Water-Soluble Low-Molecular-Weight B-(1,3-1,6)D-Glucan Isolated from *Aureobasidium pullulans* 1A1 Strain Black Yeast on Mast Cell Degranulation and Passive Cutaneous Anaphylaxis. *Bioscience, Biotechnology, and Biochemistry*, **76**, 84-88. <https://doi.org/10.1271/bbb.110536>
- [44] Żelechowska, P., Brzezińska-Błaszczak, E., Różalska, S., Agier, J. and Kozłowska, E. (2021) Native and Ige-Primed Rat Peritoneal Mast Cells Exert Pro-Inflammatory Activity and Migrate in Response to Yeast Zymosan upon Dectin-1 Engagement. *Immunologic Research*, **69**, 176-188. <https://doi.org/10.1007/s12026-021-09183-7>
- [45] Folkerts, J., Redegeld, F., Folkerts, G., Blokhuis, B., van den Berg, M.P.M., de Bruijn, M.J.W., *et al.* (2020) Butyrate Inhibits Human Mast Cell Activation via Epigenetic Regulation of FcεRI-Mediated Signaling. *Allergy*, **75**, 1966-1978. <https://doi.org/10.1111/all.14254>
- [46] Gudi, R., Suber, J., Brown, R., Johnson, B.M. and Vasu, C. (2020) Pretreatment with Yeast-Derived Complex Dietary Polysaccharides Suppresses Gut Inflammation, Alters the Microbiota Composition, and Increases Immune Regulatory Short-Chain Fatty Acid Production in C57BL/6 Mice. *The Journal of Nutrition*, **150**, 1291-1302. <https://doi.org/10.1093/jn/nxz328>
- [47] Mio, K., Ogawa, R., Tadenuma, N. and Aoe, S. (2022) Arabinoxylan as Well as B-Glucan in Barley Promotes GLP-1 Secretion by Increasing Short-Chain Fatty Acids Production. *Biochemistry and Biophysics Reports*, **32**, Article ID: 101343. <https://doi.org/10.1016/j.bbrep.2022.101343>
- [48] Petit, J., de Bruijn, I., Goldman, M.R.G., van den Brink, E., Pellikaan, W.F., Forlenza, M., *et al.* (2022) β -Glucan-Induced Immuno-Modulation: A Role for the Intestinal Microbiota and Short-Chain Fatty Acids in Common Carp. *Frontiers in Immunology*, **12**, Article 761820. <https://doi.org/10.3389/fimmu.2021.761820>