Allergy and Immunology

AIMS Allergy and Immunology, 7(1): 24–39.
DOI: 10.3934/Allergy.2023002
Received: 24 October 2022
Revised: 16 November 2022
Accepted: 02 December 2022
Published: 04 January 2023

http://www.aimspress.com/journal/Allergy

Research article

Toll-like receptor 9 is involved in the induction of galectin-9 protein by dietary anti-allergic compound fucoidan

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Abstract: Dietary intervention of fucoidan extracted from *Saccharina japonica* brown seaweed has been ascertained to favor an increase of galectin-9 protein in the intestine of allergic mice, resulting in the attenuation of the food allergy symptoms. The molecular mechanism underpinning that galectin-9 secretion remains unclear. Recently, some evidence has suggested an implication of Toll-like receptor 9 (TLR9) in galectin-9 secretion. However, no investigation has been done. For this study, we aimed to understand the relationship between galectin-9 production and fucoidan intake, which will improve the therapeutic use of fucoidan in allergy treatment. Intestinal epithelial cells (IECs) were cultured in solid or transwell plates and apically exposed to fucoidan solutions and/or synthetic TLR9 agonist (CpG-ODN). The transcriptional response of the cells to galectin-9 (lgals9) and the TLR9 gene was evaluated by using q-RTPCR, and the protein expression of galectin-9 was analyzed by conducting an ELISA test. Knockdown of TLR9 in IECs was performed by targeting TLR9 siRNA, and its effect on galectin-9 release was assessed. We found that the interaction of fucoidan and IECs resulted in the upregulation of galectin-9 released in a dose- and time-dependent manner. The increase was further potentiated in combination with the TLR9 agonist. Fucoidan exposure to IECs tended to increase the mRNA expression of TLR9 in a way similar to that of the TLR9 agonist effect, and knockdown of TLR9 in IECs resulted in a decreased tendency of fucoidan-induced galectin-9 protein. TLR9 activation is therefore involved in the increased release of galectin-9 protein observed in IECs upon fucoidan exposure.

Keywords: food allergy; dietary fucoidan; galectin-9; intestinal epithelial cells; *in vitro* study; Toll-like receptor 9

1. Introduction

"Fucoidan" is the name given to the group of fucose-rich sulfated polysaccharides extracted from the cell wall of seaweed algae of the Phaeophyceae class [1,2]. It is a complex class of macro polysaccharides with heterogeneous structures varying according to the species (variation among the same species has also been observed), the geological area of production, the season of harvest and the extraction method applied (hot-water, alkaline, enzyme-assisted extraction) [3–5]. Several studies have been conducted on its biological properties and revealed diverse bioactivities such as anti-oxidant, anti-inflammatory, anti-cancer, immunomodulatory, anti-allergic effects, ect [6–8]. Thus, increasing the interest in fucoidan in the medical research field. The anti-allergic effect of fucoidan has been depicted in numerous research studies. For instance, Herath et al. in their research, showed that oral consumption of fucoidan from *Undaria pinnatifida* attenuated allergic airway inflammation in mice by curbing key features of the allergic reaction, such as inflammatory cell infiltration, goblet cell hyperplasia, IgE production, mast cell degranulation, etc. [9]. Also, other reported anti-allergic actions of fucoidans include the promotion of the Th1 immune response over the Th2 response, increase in the number of Treg cells, impact on B cell class switching to IgE and promotion of the induction of INF-γ, IL-2 and IL-12 [10–13]. Recently, fucoidan extracted from *Saccharina japonica* was ascertained to have a protective effect on allergic mice by favoring an upregulation of galectin-9 protein in the intestine [14,15]. Galectin-9 is a lectin protein known to neutralize IgE bound on mast cells, thus preventing their degranulation and release of contents [16]. Interestingly, the authors also revealed that the anti-allergic potential only took place upon oral administration of fucoidan [14], emphasizing then the importance of the intestinal system in the induction of galectin-9 protein. However, the underpinning mechanism of fucoidan-mediated galectin-9 upregulation has not been elucidated and remains unclear.

It has been reported that fucoidan is poorly or not absorbed by the body [17,18], suggesting that receptors present on the intestinal monolayer may serve as a carrier for galectin-9 induction under its effect. Converging toward this idea, recent pieces of evidence suggest the involvement of Toll-like receptor 9 (TLR9) in the secretion of galectin-9 by intestinal epithelial cells (IECs) [19–24]. TLR9, primarily described as an endosomal receptor that recognizes specific sequences of DNA bacteria, named CpG DNA, has been revised and is nowadays found to be expressed as well at the membrane of several cell types, like human tonsil cells, splenic dendritic cells, peritoneal mast cells and IECs [25–29]. The research study results of de Kivit et al. notably showed that apical activation of TLR9 on IECs using CpG ODN (mimicking bacterial CpG DNA) induced galectin-9 release, which attenuated the food allergy symptoms in allergic mice [19,20]. Moreover, that secretion was increased in combination with non-digestible oligosaccharides [20,21]. Later, distinct reports also substantiated their observation [22–24]. Fucoidan from *Saccharina japonica* increased galectin-9 protein expression in IECs *in vivo* [14,15]. But, whether it is through the novel TLR9/galectin-9 pathway remains to be elucidated. Fucoidan is well known to be internalized by Class A scavenger receptors (SR-A) [30,31], which can trigger intracellular signaling events in cooperation with some pattern recognition receptors (PRRs) such as TLR4 and TLR9 [31]. Furthermore, fucoidan treatment has recently been shown to upregulate genes involved in the signaling of several nucleic acid-sensing receptors, such as the retinoic acid-inducible gene I-like receptor (RLR), toll-like receptor (TLR), nod-like receptor (NLR) and stimulator of interferon genes

(STING) [32], as well as to mimic DNA in solution [33]. Some fucoidans were also reported to be independent ligands of TLR2 and TLR4 [34].

Therefore, based on all the facts aforementioned, we hypothesized that TLR9 may play a role in the upregulation of galectin-9 in the intestine upon fucoidan intake. As expected, fucoidan-induced galectin-9 secretion involved TLR9 activation. Overall, this study serves to highlight the underpinning mechanism of galectin-9 upregulation consequent to fucoidan intake.

2. Materials and methods

2.1. Preparation of fucoidan

Fucoidan preparation was performed as previously described [14] with little modification. Blades of *Saccharina japonica* seaweed were ground into fine particles and stirred in sodium acetate buffer (pH 4.6) overnight at 4 ℃. The polysaccharide in the supernatant was precipitated with a large volume of absolute ethanol, collected after lyophilization and later subjected to anion-exchange chromatographic purification (Toyopearl DEAE-650M from Tosoh, Tokyo, Japan) and gel filtration chromatography (Sephacryl S-400 HR) to obtain fucoidan.

2.2. Cell culture conditions

Human IEC line, HT-29 cells purchased from ATCC (HTB-38, passages number 3-16) were cultured in a 75-cm^2 flask in McCoy's 5A medium modified (Gibco BRL, NY, USA) supplemented with 10% (v/v) fetal bovine serum (Hyclone Laboratories, UT, USA), streptomycin (100 µg/mL) and penicillin (100 U/mL). The cells were maintained in a humidified incubator at 37 ℃, 5% CO_2. At 80% confluence, the cells' medium was removed and the cells were washed with phosphate buffered saline (PBS), detached from the flask by adding trypsin and then centrifuged. The collected cells were further appropriately grown in different plates and transwells according to experimental designs. Hence, HT-29 cells were grown in 96 well plates, six well plates and 12 transwell insert filters (Corning, NY, USA) for cytotoxicity testing, transcriptional analysis and protein expression evaluation of galectin-9, respectively.

2.3. Cytotoxicity evaluation of fucoidan application on intestinal epithelial cells

The cell viability of HT-29 cells exposed to fucoidan was determined via 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay for cytotoxicity, as described by Mosmann [35], with little modification. Practically, HT-29 cells seeded in 96 well plates (100 µL of 5×10^4 cells/well) were exposed to different concentrations of fucoidan solution (0.25%, 0.5% or 1% w/v) prepared in McCoy's 5A medium for 24 hours of incubation at 37 ℃, 5% CO_2. Following stimulation of the cells with the different test samples, the fucoidan solutions were removed and MTT solution (Wako Pure Chemical Industries, Osaka, Japan) was added to the cells for 4 h of incubation. Thereafter, dimethyl sulfoxide was added to the cells after removing the MTT solution and the cells were incubated again under the same conditions for 10 min. The absorbance of the solution was measured at 570 nm with a microplate reader (SH-9000, Corona electric, Japan), and the percentage of cell viability was calculated using the formula below (Eq 1).

$$\%Cell\ Viability = \frac{(Absorbance\ of\ test - Absorbance\ of\ Blank) \times 100}{(Absorbance\ of\ cellalone - Absorbance\ of\ Blank) \times 100} \quad (1)$$

2.4. Solid plate and transwell experiments

HT-29 cells were cultured on six well-flat bottom plates. After reaching confluence, the cells were incubated with fucoidan solutions (0.05%, 0.1%, 0.2% w/v) and/or a TLR9 agonist, CpG-ODN (ODN M362, InvivoGen, USA) for 24 h. Then, the medium was removed and the cells were washed with PBS. The total RNA of the cells was extracted and q-RTPCR was performed to analyze the mRNA expression of galectin-9 and TLR9 in IECs. HT-29 cells were also cultured on 12 transwell inserts (0.4 μm; 12-mm diameter insert) and apically exposed to either fucoidan solutions (% w/v) and/or CpG-ODN (7 μM) for 24 h. The medium in the lower chamber of the transwell, referred to as a conditioned basolateral medium, was collected to analyze the protein expression of galectin-9 by applying an enzyme-linked immunosorbent assay (ELISA). In addition, the kinetics of galectin-9 released by IECs were investigated by incubating the cells with fucoidan solution at 0.1% w/v for 6, 12, 24 and 48 h.

2.5. RNA preparation and quantitative real-time polymerase chain reaction

IEC transcriptional response to galectin-9 and the TLR9 gene was analyzed by performing q-RTPCR. Cells were harvested and total RNA extraction was performed using Sepasol-RNA I Super G (Nacalai Tesque, Kyoto, Japan) coupled to chloroform/isopropanol precipitation. cDNA was synthesized by using a high-capacity cDNA reverse transcription kit (Life Technologies, Carlsbad, USA), following the manufacturer's standard protocol. The RT reaction was performed in a T100 thermal cycler (BIO-RAD, Singapore). The quantitative real-time polymerase chain reaction was performed by using a 7500 Fast-Time PCR system (Life Technologies, Carlsbad, USA) with a Fast Start Universal Probe Master (ROX) (Roche Diagnostics, Basel, Switzerland). The conditions of the PCR system were set to 95 ℃ for 10 min, 95 ℃ for 10 s, and 60 ℃ for 30 s. The TaqMan probes (Life Technologies, Carlsbad, USA) used were referenced as follows: human Galectin-9 Assay ID: Hs00371321_m1; human TLR9 Assay ID: Hs00152973_m1 and human GAPDH Assay ID: Hs99999905_m1 (housekeeping gene). Gene expressions were normalized by using GAPDH and analyzed by following a ΔΔCT quantification method.

2.6. Enzyme link immunosorbent assay

Galectin-9 protein was assessed in the conditioned basolateral media of the transwell by ELISA. Pairs of monoclonal anti-human galectin-9 antibodies from RαD system (Minneapolis, USA) were used in this experiment. High-binding-capacity ELISA plates (Greiner bio-one, Solingen, Germany) were coated with monoclonal galectin-9 capture antibodies (0.75 μg/mL) in PBS and incubated overnight at 4 ℃. The plate was then washed with PBST (PBS containing 0.05% Tween-20), blocked for 2 h with 1% bovine serum albumin (BSA) solution prepared in PBST and thereafter washed and incubated with the different test samples (basolateral conditioned medium) for 2 h. Following the incubation, and an additional washing step, biotinylated anti-human galectin-9 detection antibody (0.75 μg/mL) was added for 1 h. The plate was incubated with streptavidin-HRP

(1:500 dilution), followed by development with tetramethylbenzidine. The reaction was stopped by the addition of 2 M H_2SO_4 and the optical density was measured at 450 nm. A standard curve was performed by using recombinant human galectin-9 protein (RαD system, Minneapolis, USA).

2.7. RNA silencing of HT-29 cells with TLR9 siRNA

Silencing of TLR9 in HT-29 cells was performed as previously reported [36], with some modifications. HT-29 cells were transfected using Lipofectamine RNAiMAX reagent (Invitrogen) and TLR9 siRNA (Thermofischer, code product: 4392420) for 48 h. Briefly, the cells were transfected at 40% confluence by smooth addition of siRNA lipocomplexes at final concentrations of 25 and 50 nM; they were prepared in an OptiMEM medium (Gibco BRL, NY, USA), following manufacturer instructions. The absence of siRNA treatment (mock) was included as a control. Subsequently, the cells were stimulated with CpG-ODN (7μM) as a positive control and effective knockdown of TLR9 was confirmed in cell lysates by q-RTPCR. The same TLR9 silencing procedure was carried out in transwell experiments; the impact of TLR9 knockdown on galectin-9 release upon fucoidan was assessed by quantitative analysis of galectin-9 protein in the conditioned basolateral medium with ELISA.

2.8. Statistics analysis

Data are expressed as mean ± standard error (SE). All statistical analyses were performed by Tukey–Kramer testing. P-values below 0.05 (*$P < 0.05$) and P-values below 0.01 (**$P < 0.01$) were considered significant.

3. Results

3.1. Fucoidan is not cytotoxic for IECs at lower concentrations

The colorimetric assay of MTT analyzes the cell viability by monitoring their mitochondrial activity to metabolize MTT product into formazan, a purple derivative. Cytotoxicity evaluation of fucoidan on HT-29 cells assessed by MTT assay showed no toxicity at concentrations below 1% w/v (Figure 1). Therefore, fucoidan concentrations below 1% w/v were selected for the following experiments.

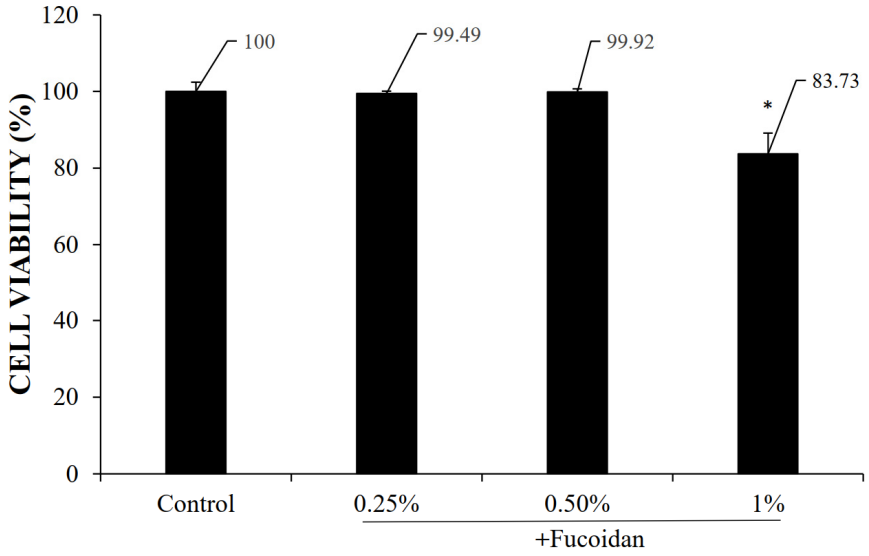

Figure 1. Effect of fucoidan on IEC cell viability. IECs (HT-29 cells) were treated with 0.25%, 0.5% and 1% w/v fucoidan for 24 h. Cell viability was determined by conducting an MTT assay. Data are presented as mean ± SE (n = 3). *P < 0.05 by Tukey–Kramer analysis.

3.2. Fucoidan increases gene expression of galectin-9 and favors its protein release by IECs in a dose- and time-dependent manner

It was reported that oral administration of fucoidan to mice favored galectin-9 protein induction *in vivo*, which was a paramount mechanism to the anti-allergic effect of the fucoidan extract [14,15]. Indeed, the intravenous injection of anti-galectin-9 antibody to fucoidan-fed-allergic mice abrogated fucoidan's suppression of the allergic symptoms in mice. Moreover, the anti-allergy effect took place only when the mice received fucoidan by gastric gavage [14,15], emphasizing the important role played by the intestinal system in galectin-9 induction. Studies have shown that fucoidan, because of its physicochemical properties, can hardly be degraded by intestinal enzymes and can therefore be present in all its integrity in the lumen of the intestinal tract with good stability [37]. Therefore, to investigate the induction of galectin-9 *in vitro*, HT-29 cells were apically exposed to different concentrations of fucoidan solution (Figure 2). The galectin-9 mRNA (*lgals9*) level tended to increase upon direct exposure to fucoidan (Figure 3a), albeit not significantly. IECs are at the cross-borders between dietary foods in the lumen and immune cells present in the lamina propria. This bi-interface of IECs was mimicked here by using transwell plates, where IECs were seeded in an apical side of the transwell, and fucoidan solutions were apically applied to them while the lower chamber was filled with the medium (basolateral medium). In the conditioned basolateral mediums, galectin-9 protein expression significantly increased with increasing concentration of fucoidan applied to the cells, showing a dose-dependent manner (Figure 3b). In addition, the time of fucoidan stimulation on IECs affected the secretion of galectin-9. An increase in the time was noted to favor a higher release of galectin-9 by IECs (Figure 3c). Taken together, these data suggest that stimulation with a fucoidan concentration of 0.1% w/v for 24 h is the optimal condition for observing the effect of fucoidan on

galectin-9 secretion by IECs. It was ascertained that fucoidan can directly promote galectin-9 induction in IECs.

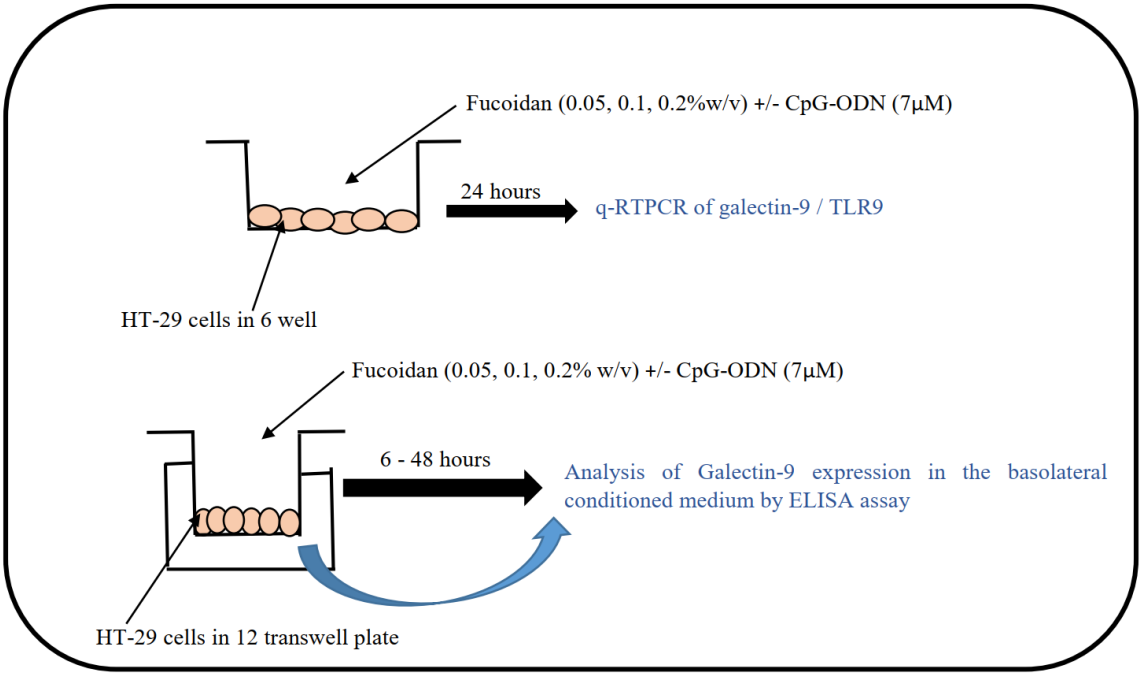

Figure 2. Schematic depiction of *in vitro* experiments. HT-29 cells cultured in flat-bottom plates were incubated with fucoidan solution at 0.05, 0.1, or 0.2% w/v for 24 h. Cells were harvested and the total RNA was extracted. Transcriptional response of the cells to fucoidan was evaluated by applying q-RTPCR testing for the galectin-9 gene (Lgal9). The cells were also grown on transwell insert filters and apically stimulated with fucoidan solution (0.05, 0.1, 0.2% w/v) for 6, 12, 24 and 48 h. Galectin-9 in the conditioned basolateral medium was measured by ELISA test.

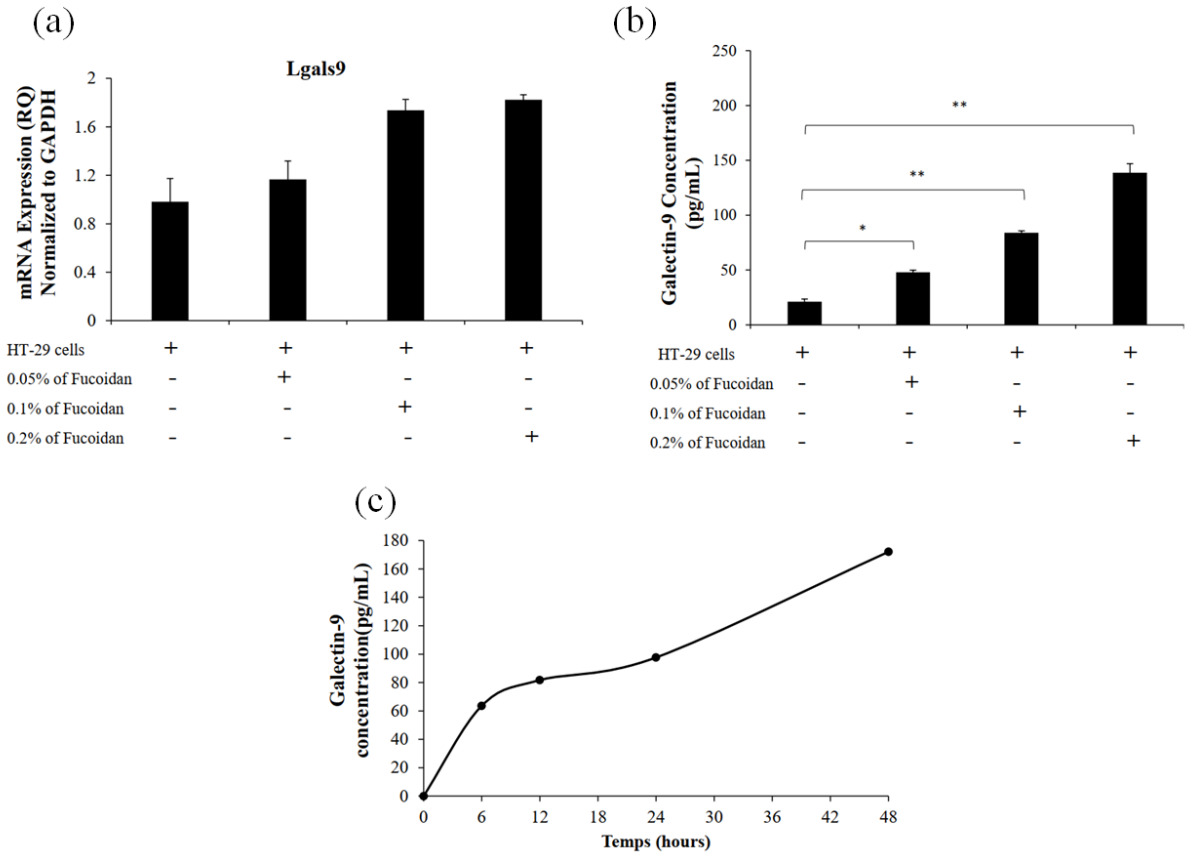

Figure 3. Fucoidan directly acts on IECs and favors galectin-9 released in a dose- and time-dependent manner. Fucoidan solution, at different concentrations, was used to directly stimulate HT-29 cells at several defined times (confer Figure 2). (a) mRNA gene level of galectin-9 expressed by HT-29 cells after fucoidan treatment (24 h). (b) Dose response of galectin-9 secretion by HT-29 cells upon fucoidan exposure (24 h). (c) Kinetics of galectin-9 upregulation in fucoidan-stimulated IECs. Data are presented as mean ± SE (n = 3). *P < 0.05; **P < 0.01 by Tukey–Kramer test.

3.3. Synergetic effect of fucoidan and CpG-ODN in galectin-9 released by IECs

Fucoidan is prebiotic; it has been shown to impact gut microbiota [38,39]. And, recently, it was evidenced that galectin-9 secretion by IECs is involved in the immunomodulatory effect of several prebiotics, such as short-chain galacto-oligosaccharides/long-chain fructo-oligosaccharides and 2'-fucosyllactose, in synergetic action with bacterial DNA [20–24]. In *in vitro* studies, bacterial DNA is often mimicked by the use of CpG-ODN, resembling the palindromic CpG-DNA sequence, a cytosine-phosphate-guanine dinucleotide (CpG) motif found abundantly in bacterial DNA, which is responsible for the immunostimulatory activity of probiotics [40]. To evaluate a plausible synergetic effect of fucoidan and bacteria trigger (mimicking *in vitro* by the use of CpG-ODN) in the induction of galectin-9, the cells, seeded in a transwell plate, were apically exposed to either fucoidan solution (0.1%) or CpG-ODN (7μM) alone, or to a combined solution of fucoidan and CpG-ODN, for 24 h. Apical ligation of TLR9 on IECs by its ligand CpG-ODN resulted in a significant upregulation of

galectin-9 protein. The combined exposure of CpG-ODN and fucoidan twice induced the release of galectin-9 protein, showing a synergetic effect (Figure 4).

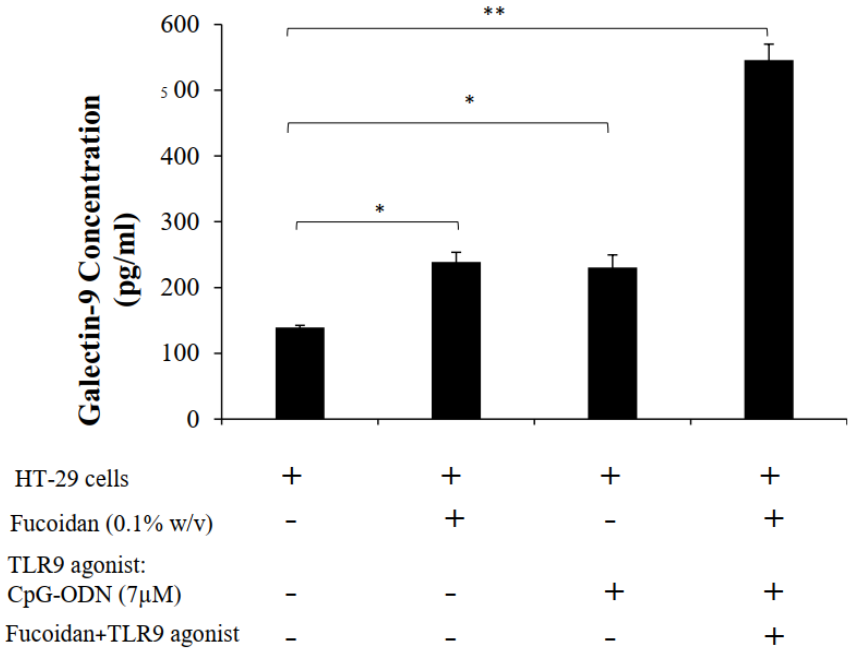

Figure 4. Synergetic effect of fucoidan and TLR9 agonist in terms of inducing galectin-9 secretion by IECs. IECs cultured in transwell inserts were apically exposed to fucoidan and/or TLR9 agonist (CpG-ODN) for 24 h. Galectin-9 protein in the basal medium was analyzed by ELISA test. Combined exposure of fucoidan and TLR9 agonist synergistically potentiated galectin-9 protein release. Data are presented as mean ± SE (n = 3). *P < 0.05; **P < 0.01 by Tukey–Kramer test.

3.4. Tendency of increased TLR9 mRNA level in IECs following fucoidan exposure and of decreased galectin-9 released after knockdown of TLR9

To examine the role of TLR9 in fucoidan-mediated galectin-9 release, we first evaluated the expression of TLR9 in IECs following fucoidan exposure. The mRNA expression of TLR9 in IECs tended to increase upon apical fucoidan stimulation. Likewise, stimulation of the cells with CpG-ODN, a TLR9 agonist, tended to increase the TLR9 mRNA expression (Figure 5a). Effective knockdown of TLR9 using TLR9 siRNA was established with a 40% decrease in the mRNA expression of TLR9 as compared to the positive control cells (cells stimulated with TLR9 agonist) (Figure 5b). To assess the impact of TLR9 knockdown on fucoidan-mediated galectin-9 upregulation, transfected cells with TLR9 siRNA were subsequently stimulated with fucoidan and galectin-9 protein in the conditioned basolateral medium; the result was quantified by ELISA. A decreased tendency of galectin-9 released as an effect of fucoidan was observed, showing an implication of TLR9 in fucoidan-mediated galectin-9 upregulation on IECs (Figure 5c).

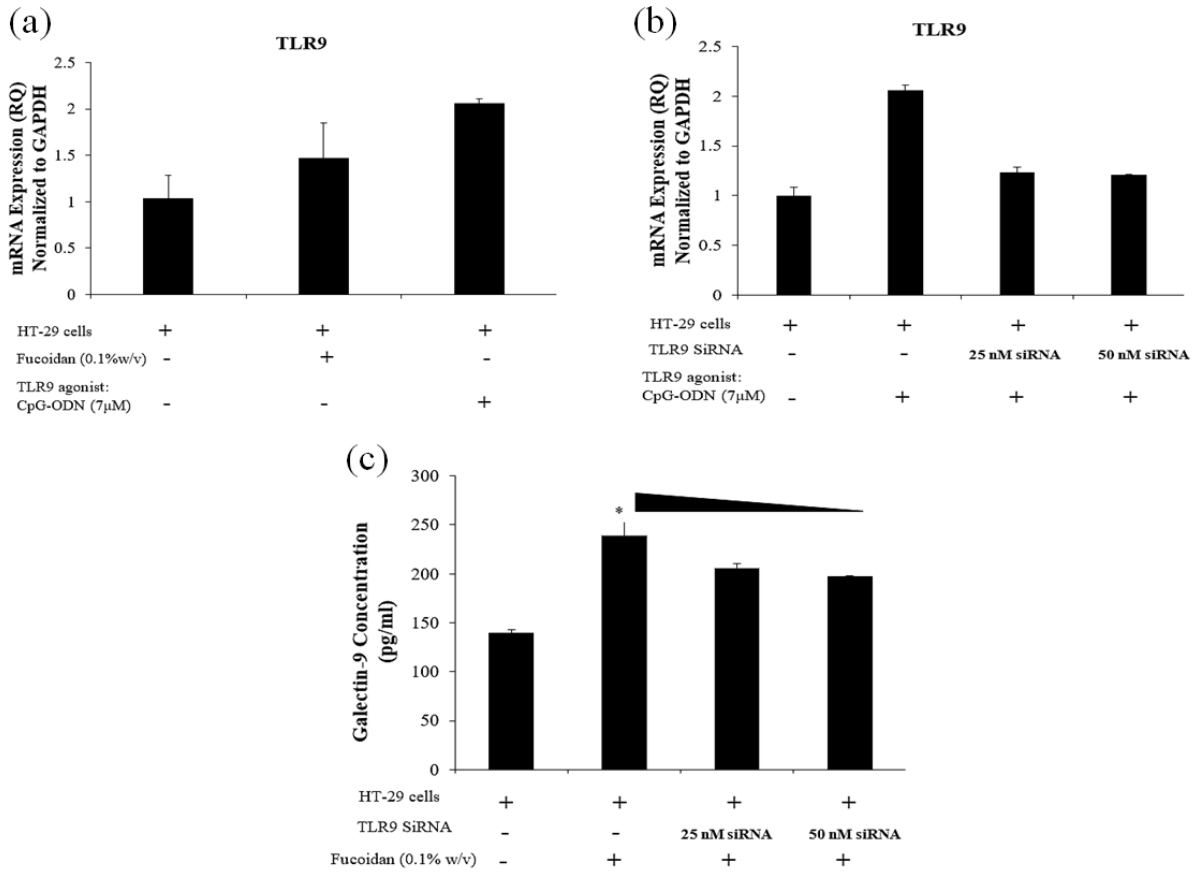

Figure 5. Fucoidan-upregulated TLR9 mRNA expression in IECs and knockdown of TLR9 decreased the level of galectin-9 protein. (a) mRNA gene level of TLR9 expressed by HT-29 cells after fucoidan and TLR9 agonist stimulation for 24 h. (b) Knockdown of TLR9 in HT-29 was performed by using TLR9 siRNA at 25 nM and a 50 nM final concentration. (c) Knockdown of TLR9 on HT-29 cells further resulted in a gradual decrease of galectin-9 secretion induced by fucoidan. Data are presented as mean ± SE (n = 3). *P < 0.05 by Tukey–Kramer test.

4. Discussion

The fucoidan contained in *Saccharina japonica* is composed of fucose and sulfated fucose [15]. Preceding studies have demonstrated *in vivo* the induction of galectin-9 protein in the intestine following oral intake of fucoidan [14,15]. In this novel study, the induction of galectin-9 by fucoidan was assessed *in vitro*. Consistently, galectin-9 released by IECs significantly increased upon fucoidan stimulation, suggesting that, when reaching the intestine, fucoidan may directly interact with the intestinal epithelium cells, resulting in the upregulation of galectin-9 protein. Moreover, the increase in the dose and time of exposure resulted in a higher release of galectin-9, highlighting a dose- and time-dependency effect. These two parameters should therefore be taken into account in fucoidan therapeutic strategies.

TLR9 belongs to the family of pattern recognition receptors and is expressed on endothelium, epithelium and immune cells [41]. Its canonical pathway to activation is reported to first involve the

recruitment of myeloid differentiation primary response 88 adaptor protein to the TIR domain. This initiates a cascade event comprising the engagement of further adaptor proteins, such as IRAKs, TRAFs and IKKs, leading to activation of transcription factors NF-κβ, AP-1 and IRF-7, as well as the induction of either proinflammatory molecules or type I interferons [42,43]. In IECs, though, the signaling of TLR9 appears to be more complex. As such, Lee et al. observed that chloroquine and bafilomycin A1, which are distinct endosomal inhibitors that neutralize the acidic pH that is vital for the proper maturation of TLR9 endosomes, were ineffective in impeding TLR9-mediated activation of NF-κβ in IECs [25]. This fact pointed out the existence of a signaling pathway that is different from the aforementioned pathway for IECs. In addition, surface expression of TLR9 at similar levels was confirmed by different means (flow cytometry, confocal microscopy imaging and vectorial biotinylation) at both the apical and basolateral faces of IECs. Apical activation directed an anti-inflammatory effect, while the latter promoted an inflammatory response [25].

Recently, a non-canonical TLR9/galectin-9 pathway in IECs has emerged, opening new perspectives on the functionality of this receptor and challenging the amphoteric knowledge on this receptor. The fucoidan induction of galectin-9 in this study, upon apical stimuli of IECs, was associated with a trendy increase of TLR9 mRNA expression similar to the effect of the TLR9 agonist. In addition, the apical ligation of TLR9 on IECs by its agonist also favored the secretion of the galectin-9 protein, which was even potentiated by combined exposure with fucoidan. These results are consistent with several reports unveiling a synergetic effect of a TLR9 agonist with other non-digestible oligosaccharides in the galectin-9 released by IECs [20–24]. The precise mechanism beyond the synergetic effect is, to date, unclear, but, regarding fucoidan, several plausible explanations could be formulated. Some reports mentioned that fucoidan can be internalized by SR-A, which may trigger the intracellular signaling of some PRRs, notably TLR9 [31]. In that line, a recent study showed that fucoidan's treatment upregulated genes involved in the signaling of several nucleic acid-sensing receptors, such as RLR, TLR, NLR and STING [32]. Moreover, it is not excluded that fucoidan may interact directly with TLR9, independently of an association with SR-A. Yamazaki et al. showed that fucoidan mimics DNA in solution [33], and Makarenkovaa et al. showed fucoidan polysaccharides to be independent ligands of TLR2 and TLR4 [34]. In this study, we observed that increasing the knockdown of TLR9 on HT-29 cells further resulted in a gradual decrease of galectin-9 secretion and tended to downregulate the upregulation observed upon fucoidan exposure. Taken together, these results strongly imply that TLR9 is involved in the increase in galectin-9 and basal secretion upon exposure to fucoidan on IECs. Knowledge of TLR9 is far from complete, and the complexity of this receptor, linked to intense variability in its cellular localization (intracellular or surface expression), cell trafficking, cell-type dependency and even trigger-type dependency [44,45] have just begun to be analyzed.

5. Conclusions

The results of this study provide new insight into how fucoidan potentiates galectin-9 increase in mice, as well as completes the understanding of the fucoidan anti-allergic mechanism. A graphical overview is notably presented below (Figure 6). Galectin-9 upregulation appeared to result from both the direct interaction of fucoidan with IECs and a synergetic effect with probiotic DNA. In addition, this study is a piece of additional evidence for the existence of the TLR-9/galectin-9 pathway in IECs, which stands as a promising path for the resorption of allergy disease. Advances in molecular

biology techniques will surely allow for deeper investigations into TLR9 signalization on IECs leading to galectin-9 upregulation.

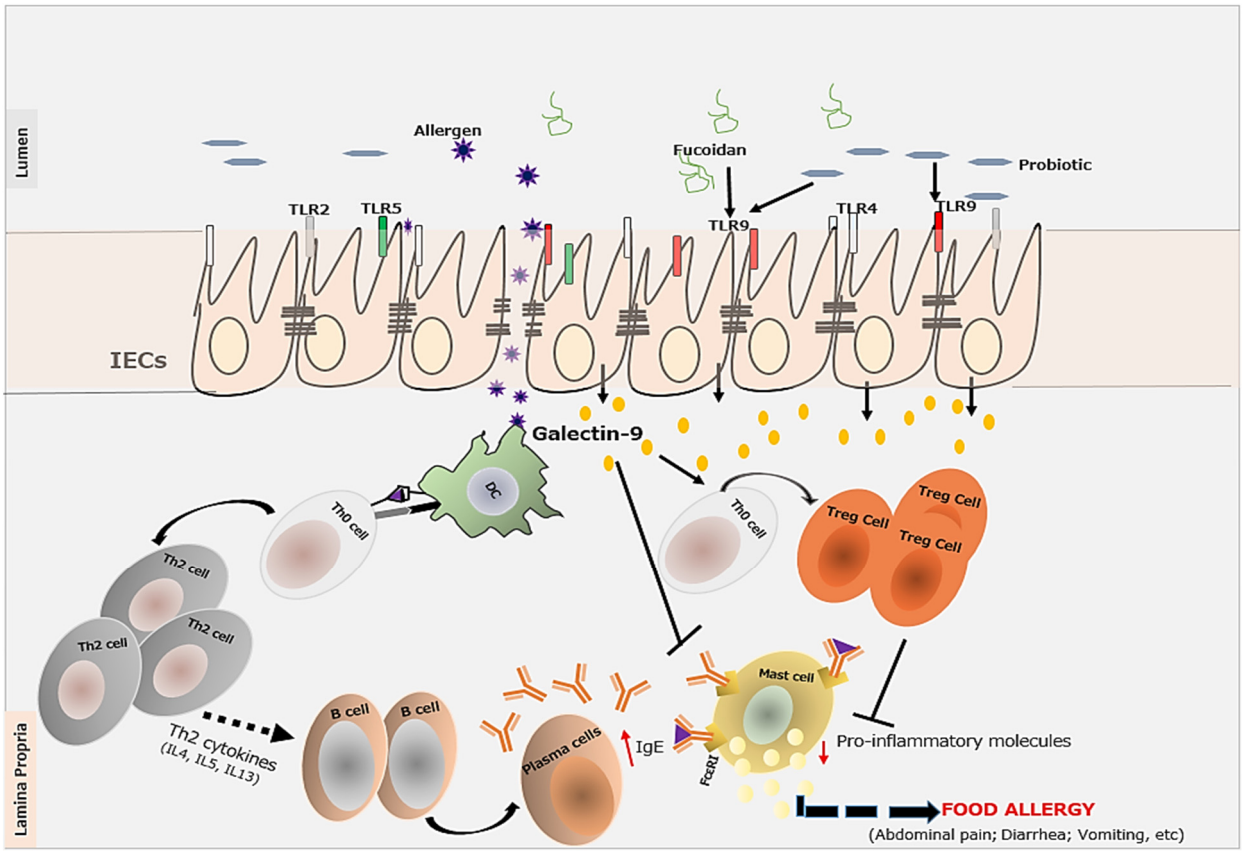

Figure 6. Graphical overview of fucoidan-mediated galectin-9 upregulation in the intestine, and the effect of galectin-9 on food allergy resorption. When reaching the intestine, fucoidan interacts with the TLR9 expressed by IECs and synergize with beneficial probiotics to induce galectin-9 release by IECs. Galectin-9 protein prevents mast cell degranulation by neutralizing or removing the IgE bound on their surface. It can also induce the polarization of naïve T cells to regulatory T cells (Treg), which contributes to preventing mast cell degranulation. Thus, the release of proinflammatory mediators from mast cell granules is dampened and, consequently, the food allergy symptoms are attenuated.

Acknowledgments

The study was supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan through the MEXT scholarship program and JSPS KAKENHI Grant Number JP18K19741.

Conflict of interest

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

Author contributions

Conceptualization: E.E and M.M; Methodology: E.E and M.M; Manuscript draft: E.E; Manuscript review and approval: E.E and M.M.

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