Reduced Expression of Polymeric Immunoglobulin Receptors in the Intestine of Young Rats Fed a Fiber-free Diet

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In this study, we investigated the influence of a fiber-free diet on the intestinal secretory immune system in young animals. Four-week-old rats were fed either a purified diet containing sucrose as the only carbohydrate source (fiber(−) diet) or a diet supplemented with 15% natural crude fiber from sugar beets (fiber(+) diet). After 14 days of feeding, we measured total IgA content in 24-hr fecal samples and in intestinal tissues and the expression of intestinal polymeric immunoglobulin receptors (pIgRs), which are essential for IgA secretion. The excretion of total IgA in the feces was significantly lower in rats fed the fiber(−) diet than in those fed the fiber(+) diet (27% vs. 100%; $p < 0.05$). However, the total IgA content in the intestinal tissue extracts did not differ between the groups. The pIgR signal intensities observed by immunohistochemistry were somewhat lower in the colon of the rats fed the fiber(−) diet. Western blot analysis showed that pIgR protein expression in the distal colon of rats fed the fiber(−) diet was significantly lower than that in rats fed the fiber(+) diet (38% vs. 100%, $p < 0.05$). Conversely, colonic pIgR mRNA expression did not differ between the groups. Thus, we conclude that a fiber-free diet decreases colonic pIgR protein expression by a posttranscriptional mechanism, resulting in decreased luminal secretory immune system activity and thus, suboptimal protection of the colonic mucosa.

Key words: secretory immune system; immunoglobulin A; polymeric immunoglobulin receptor; fiber-free diet; rat

INTRODUCTION

The secretory immune system is a unique, local immunological mechanism that is independent of systemic immunity. Secretory immunoglobulin A (SIgA), a major component of the mucosal immune system, can inhibit initial pathogen colonization via immune exclusion, both on the mucosal surface and within virus-infected secretory epithelial cells [1]. In the intestinal lamina propria, polymeric IgA (pIgA, mainly dimers) is produced by IgA-secreting plasma cells and subsequently endocytosed, transported, and secreted into the lumen by epithelial cells. The polymeric immunoglobulin receptor (pIgR), also known as the membrane secretory component (SC), is an integral membrane protein expressed by intestinal epithelial cells. The physiological role of pIgR is to bind and transport J-chain-containing pIgA antibodies across the

intestinal epithelial cell layer and to protect them from proteolytic degradation in the secretions [2]. Thus, the intestinal secretory immune system is generated by a unique cooperation between 2 distinct cell-types: lymphatic plasma cells that produce pIgA and nonlymphatic epithelial cells that express pIgR.

It is well known that oral administration of total parenteral nutrition (TPN) in experimental animals leads to increased bacterial translocation [3]. Spaeth et al. [4, 5] reported that in a study using rats, TPN decreased the SIgA content in small intestinal lavage and IgA content in the small intestinal tissue. Similarly, in a study by Sano et al. using mice [6, 7], oral administration of TPN decreased the SIgA content in small intestinal lavage and/or pIgR expression in the small intestinal tissue. Since TPN is a fiber-free diet, these studies suggest that a fiber-free diet leads to a suboptimal state of the secretory immune system in the intestine. However, these studies used the Chow diets as the comparative control. The Chow diets had different nutritive compositions than TPN. Accordingly, in these studies, the evaluation of the effects of a fiber-free diet on the secretory immune

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system of the intestine was not on the basis of consistent nutritive compositions. Conversely, Spaeth et al. used either TPN [4] or a total liquid diet (a nutrient-defined fiber-free diet) [5] as the fiber-free diets, and the comparative diets were these same diets with added bulk fiber (cellulose). However, these studies found no difference in either the SIgA content in small intestinal lavage or in the IgA content in the intestinal tissue between the fiber-containing diet and fiber-free diet groups. For this reason, much remains unknown about how a fiber-free diet can affect the secretory immune system of the intestine.

Therefore, in the present study, we compared the IgA content in 24-hr fecal samples, the IgA content in intestinal tissue, and the levels of pIgR expression (mRNA and protein) in the intestinal tissue of young rats (4-week-old) fed either a fiber-free diet (fiber(–) diet) with sucrose as the only carbohydrate source or a fiber-containing diet (fiber(+) diet) formulated with dietary fiber from beets, which is a naturally occurring dietary fiber.

MATERIALS AND METHODS

Animals and diets

Male Wistar/ST rats (4 weeks old, Japan SLC, Shizuoka, Japan) were maintained in individual wire-mesh cages without bedding material in a temperature-controlled (23 ± 1°C) room under a 14-hr light cycle (light: 0500–1900 hr). The composition of the fiber(–) diet is shown in Table 1. The mineral mixture was identical to MM2 formulated by Ebihara et al. [8], and the vitamin mixture was prepared in accordance with the AIN-76 mixture [9], except that menadione and L-ascorbic acid were added to 5.81 μmol/kg [10] and 284 μmol/kg [11], respectively. The fiber(+) diet was prepared by replacing 15% (w/w) of the fiber(–) diet with beet dietary fiber (Nippon Beet Sugar Manufacturing, Obihiro, Japan). According to Aritsuka et al. [12], the composition of beet dietary fiber is as follows (g/100 g): moisture, 4.5; total dietary fiber, 81.1 (cellulose, 23.0; hemicellulose, 22.0; pectin, 19.0; lignin, 3.0; unidentified matter, 14.4); protein, 9.0; lipid, 0.6; sucrose, 1.5; and ash, 3.0. The rats were divided into 2 groups (*n* = 6/group) on the basis of body weight. They were fed either the fiber(–) or fiber(+) diet for 14 days and were allowed free access to food and water throughout the experimental period.

The study was approved by the Animal Use Committee of Hokkaido University, and the animals were maintained in accordance with the Guidelines for

the Care and Use of Laboratory Animals of Hokkaido University.

Fecal sample preparation

The fecal samples were prepared as described previously [13]. On day 14 of the experiment, fecal samples were collected for 24 hr from each rat, lyophilized and mixed with 50 volumes of phosphate-buffered saline (PBS) containing 100 μg/ml of soybean trypsin inhibitor. The samples were then homogenized on ice by using a Polytron homogenizer (30 sec, 3 times) and centrifuged at $1,600 \times g$ for 15 min. The supernatant was used for quantification of fecal IgA.

Preparation of tissue samples

On the last day of the feeding period, the rats were sacrificed using an intraperitoneal injection of a solution of ketamine hydrochloride (70 mg/kg body weight; Wako Pure Chemical Industries, Osaka, Japan) and xylazine hydrochloride (8 mg/kg body weight; ICN Biomedicals, Aurora, OH, USA), and their intestines were carefully removed. The luminal contents were flushed out with ice-cold PBS. The small intestine from the Treitz ligament to the ileocecal junction was divided into 2 equal segments. The proximal and distal halves were designated as the jejunum and the ileum,

Table 1. Fiber-free diet (fiber(–) diet) composition[1]

Ingredient	g/kg Diet
Casein[2]	250
Corn oil[3]	50
Mineral mixture[4]	40
Vitamin mixture[5]	10
Choline chloride[6]	2
Vitamin E granules[7]	1
Cholecalciferol[8]	0.0025
Sucrose[9]	To make 1 kg

[1] Fiber(+) diet was prepared by replacement of 15% (w/w) of fiber(–) diet with beet dietary fiber (Nippon Beet Sugar Manufacturing, Obihiro, Japan).
[2] Casein (Edible Acid Casein; Murray Goulburn Co-operative, Victoria, Australia).
[3] Retinyl palmitate (7.66 μmol/kg diet) was added to corn oil.
[4] The mineral mixture was identical to MM2 formulated by Ebihara et al. [8].
[5] The vitamin mixtures were prepared according to the AIN-76 mixture [9], except that menadione and L-ascorbic acid were added to a total of 5.81 μmol/kg [10] and 284 μmol/kg [11] diet.
[6] Four milliliters of choline chloride solution (3.58 mol/l) in 50% (v/v) ethanol was added per kg diet.
[7] The trade name is "Juvela granule" (Eisai); this contained 423 μmol all-*rac*-α-tocopheryl acetate per kg diet.
[8] Vitamin D_3 (crystal, 400,000 IU/g).
[9] Granulated beet sugar was supplied by Nippon Beet Sugar Manufacturing, Obihiro, Japan.

respectively. The colon, excluding the cecum, was divided into 2 equal segments and defined as the proximal and distal colons. A 1-cm segment was excised from the middle of the jejunum, proximal colon, and distal colon. These were then embedded in OCT compound (Miles Scientific, Elkhart, IN, USA), frozen in liquid nitrogen, and stored at $-80°$C for immunohistochemical analysis. Similarly, a 1-cm segment was excised from the proximal and distal colons, and the mucosa of the segment was scraped off with a glass slide for total RNA extraction. The mucosa of the remaining segment was scraped off with a glass slide, and 20 volumes of 50 mmol/l Tris-HCl (pH 7.4) containing 1 mmol/l phenylmethylsulfonyl fluoride, 5 mmol/l EDTA, 100 μg/ml soybean trypsin inhibitor, 100 μg/ml leupeptin, and 100 KIU/ml aprotinin were added. The tissue was homogenized on ice by using a Polytron homogenizer (Kinematica AG, Littau, Switzerland), and an aliquot of the homogenate was centrifuged at 10,000 $\times$ g for 15 min. The supernatant was used for quantification of intestinal IgA. Intestinal plasma membranes were prepared from the homogenate, according to the method described by Ahnen et al. [14], and used for quantification of pIgR by Western blot analysis. Briefly, an aliquot of the intestinal homogenate was centrifuged at 750 $\times$ g for 10 min to remove cells and nuclei. Membranes were pelleted from the supernatant by centrifugation at 20,000 $\times$ g for 20 min, resuspended, and boiled for 5 min in Laemmli sample buffer for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Total IgA determination

Total IgA concentration was measured using an enzyme-linked immunosorbent assay. At room temperature, 96-well microtiter plates (Nunc, Roskilde, Denmark) were coated with 100 μl of goat anti-rat IgA antibodies (Bethyl Laboratories) (1:100) dissolved in PBS. The unbound antibodies were removed by 3 washes with 125 μl of PBS containing 0.05% (v/v) Tween-20 (PBS-T). The plates were incubated with 125 μl of 1% (w/v) bovine serum albumin (BSA) for 30 min at room temperature and washed with PBS-T 3 times. Intestinal tissue extracts, fecal samples, or standard rat IgA (Bethyl Laboratories) was diluted with PBS. The diluted samples or standards were added to the wells in triplicate. For each sample, an uncoated well blocked with 1% BSA was used as the control for nonspecific binding. After 1-hr incubation at room temperature, the plates were washed, and 100 μl of horseradish peroxidase-conjugated goat anti-rat IgA antibodies

(Bethyl Laboratories) (1:50,000) was added. Following further incubation at room temperature for 1 hr, the plates were washed, and 100 μl of 3,3′,5,5′-tetramethylbenzidine substrate reagent (BD Biosciences Pharmingen, San Diego, CA, USA) was added. After incubation at room temperature for 15 min, color development was stopped by adding 50 μl of 1 mol/l H_2SO_4, and the absorbance at 450 nm was measured. The total IgA concentration of each sample was calculated using a standard curve.

Histological observation of polymeric immunoglobulin receptor expression in the intestine

Frozen sections (6 μm) were prepared with a cryostat (Minotome; International Equipment Co., Boston, MA, USA), thawed onto poly-L-lysine-coated glass slides, and immediately dried with a hair dryer. The sections were fixed with 4% (w/v) paraformaldehyde in PBS for 10 min, followed by treatment with methanol containing 3% (w/v) H_2O_2 for 10 min. The slides were washed 3 times with PBS and then pre-blocked with PBS containing 10% (w/v) normal goat serum (Cedarlane Laboratories, Burlington, ON, Canada), 3% (w/v) casein Na, and 1% (w/v) BSA (Intergen, Purchase, NY, USA) (buffer 1) at 37°C for 30 min. After washing with PBS, the slides were incubated at 37°C for 90 min in a 1:400 dilution of rabbit polyclonal antibodies against human pIgR, which cross-reacts with rat pIgR (Nordic Immunological Laboratories, Tilburg, The Netherlands) in buffer 1. They were then incubated at 37°C for 60 min in a 1:400 dilution of biotin-conjugated goat anti-rabbit IgG antibodies (Zymed Laboratories, South San Francisco, CA, USA) in buffer 1. The slides were washed 3 times with PBS and then incubated at 37°C for 30 min in a 1:500 dilution of horseradish peroxidase-conjugated streptavidin (Research Diagnostics, Inc., Flanders, NJ, USA) in buffer 1. After washing with PBS, the slides were incubated for 5 min at room temperature in 50 mmol/l Tris-HCl (pH 7.4) containing 0.05% (w/v) 3,3′-diaminobenzidine and 0.015% (w/v) H_2O_2. The nuclei were counterstained with hematoxylin.

Immunoblotting for intestinal polymeric immunoglobulin receptor quantification

The intestinal pIgR content was measured by Western blot analysis. The plasma membranous fraction of the intestinal mucosa was subjected to 7.5% SDS-PAGE under reducing conditions [15]. The separated proteins were transferred electrophoretically to a nitrocellulose membrane (Hybond-C Extra, Amersham International Plc, Bucks, UK) in a semi-dry electroblotting apparatus

(Nihon Eido, Tokyo, Japan) at a constant current of 150 mA for 1 hr. The blotting buffer contained 125 mmol/l Tris and 960 mmol/l glycine in 20% (v/v) methanol (pH 8.3). The membrane was then incubated in a blocking solution of 5% (w/v) skimmed milk powder in Tris-buffered saline (20 mmol/l Tris-HCl, 150 mmol/l NaCl, pH 7.4), followed by incubation for 1 hr with a 1:2,000 dilution of rabbit polyclonal antibodies against rat pIgR (Bethyl Laboratories, Montgomery, TX, USA) in a blocking solution containing 0.05% (v/v) Tween-20. The membrane was then washed 3 times with Tris-buffered saline containing 0.05% (v/v) Tween-20. Next, the membrane was incubated for 1 hr with a 1:5,000 dilution of horseradish peroxidase-conjugated goat anti-rabbit IgG antibodies (Biomedical Technologies, Stoughton, MA, USA) followed by washing in the same manner as that after the primary antibody. Identification of the antigen-antibody complex was performed using Renaissance Western Blot Chemiluminescence Reagent (DuPont NEN Research Products, Boston, MA, USA), as recommended by the manufacturer, except that the exposure to the x-ray film was for 15 min. The relative values of pIgR were estimated by densitometric scanning (Dual-Wavelength CS-9000 Flying-Spot Scanner, Shimadzu, Kyoto, Japan). The values were normalized relative to the average value of the fiber(+) diet group, which was set to 100%. Molecular weight markers (Bio-Rad Laboratories, Hercules, CA, USA) were used on each gel, and a liver plasma membrane sample from a 10-week-old rat was used as the positive control.

RNA isolation and Northern blot analysis

Intestinal pIgR mRNA expression was measured by Northern blot analysis. Total RNA was isolated by ISOGEN (Nippon Gene, Tokyo, Japan), according to the manufacturer's protocol. The samples of total RNA (25 µg/lane) were electrophoresed on a denaturing 2.2 M formaldehyde 1% agarose gel and transferred to a nylon membrane (Hybond N+, Amersham). Blots were hybridized with a 54-base oligonucleotide probe (5´-AGCGTCCATTCTCGTCGGCTTCCCAGTGACACC AGTACTTGAGGCTGCTGCTTT-3´), which was complementary to rat pIgR mRNA. The probe was 3´-labeled using a non-radioisotopic system, a DIG Oligonucleotide Tailing Kit (Boehringer Mannheim, Mannheim, Germany). Prehybridization, hybridization, and detection were performed with a DIG-Luminescent Detection Kit (Boehringer Mannheim), as recommended by the manufacturer. The relative quantity of pIgR mRNA was estimated by densitometry scanning. The value of pIgR mRNA was normalized to the value of 18S ribosomal RNA.

Statistical analysis

The experimental data were expressed as mean + standard deviation (SD) values of 6 rats per group. Differences were evaluated using the Student's *t*-test with the StatView 4.0 program (Abacus Concepts Inc., Berkeley, CA, USA). Differences were considered to be significant for *p*-values <0.05.

RESULTS

Body weight did not differ between the groups fed the fiber(−) and fiber(+) diets throughout the experiments (data not shown). The dry fecal weight and total IgA content in the 24-hr fecal samples were significantly lower in the rats fed the fiber(−) diet than in those fed the fiber(+) diet (Table 2). The total IgA content in the intestinal tissue extracts (jejunum, ileum, proximal colon, and distal colon) did not significantly differ between the groups (Fig. 1).

To determine the cellular pattern of pIgR protein expression, immunohistochemical analysis of intestinal pIgR protein in rats fed the fiber(−) and fiber(+) diets was performed. In the jejunum, the pIgR protein signals were clearly detected in the mucosal epithelium at the crypt regions of both groups, and the signal intensities were not different between the groups (Fig. 2, A and B). The pIgR signals were also detected in the crypt epithelia of the proximal and distal colons. The signal intensities appeared somewhat lower in rats fed the fiber(−) diet than in those fed the fiber(+) diet (Fig. 2, C vs. D and E vs. F).

Fig. 3a shows representative immunoblots of pIgR protein in the jejunum (lane 1) , ileum (lane 2), proximal colon (lane 3) and the distal colon (lane 4) of rats fed the fiber(+) diet and in the liver plasma membranes of a 10-week-old rat used as a positive control (lane 5). In the liver plasma membrane, pIgR was present as a doublet (108,000 and 102,000 MW), whereas in the jejunum, ileum, proximal colon and distal colon, it was found as a single band (91,000, 91,000, 99,000 and 99,000 MW, respectively). These results are consistent with those of previous reports that investigated the posttranslational processing of pIgR protein by endopeptidase in the rat small intestines [14, 16]. We estimated the relative quantities of pIgR protein in rats fed the fiber(−) and fiber(+) diets by densitometric scanning.

The amount of pIgR protein was significantly lower in the distal colon of the rats fed the fiber(−) diet than in those fed the fiber(+) diet (38% vs. 100%; *p* < 0.05) (Fig. 3b). Although not statistically significant, the amount of

Table 2. Dry fecal weight and total IgA content in 24-hr fecal samples of rats fed fiber(–) and fiber(+) diets[1]

	Fiber(–) diet		Fiber(+) diet	
	Mean	SD	Mean	SD
Dry fecal weight (g)	0.27*	0.10	1.14	0.32
Total IgA content (μg)	168*	145	620	419

[1] The fiber(+) diet includes 15% (w/w) beet dietary fiber (Nippon Beet Sugar Manufacturing, Obihiro, Japan). For details of the fiber(–) diet, see Table 1. *A significant difference from the fiber(+) diet group ($p < 0.05$) was observed using the Student's t-test.
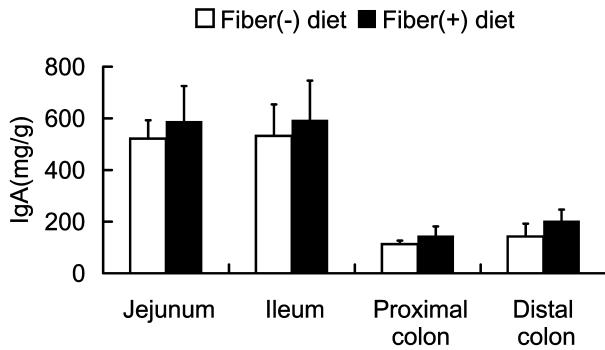

Fig. 1. Total IgA levels in the intestinal tissue extracts of rats fed the fiber(–) and fiber(+) diets. The results are expressed as mean + SD values ($n = 6$).
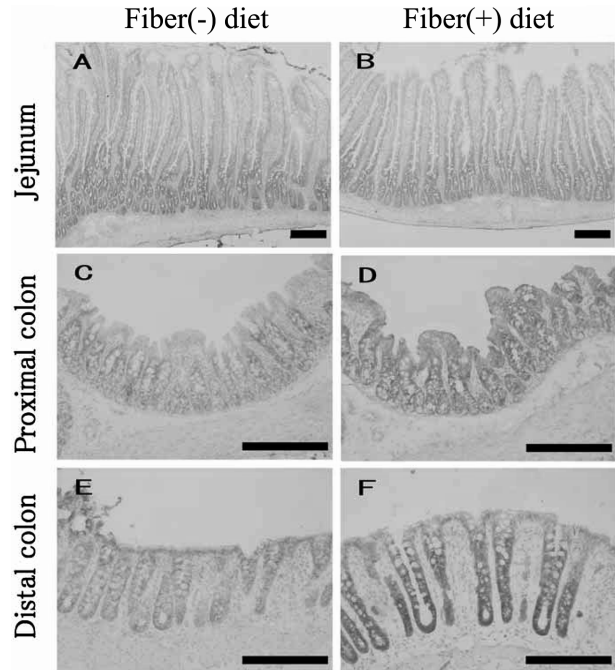

Fig. 2. Immunohistochemical analysis of intestinal pIgR protein. Four-week-old rats were administered a fiber(–) diet (A, C, E) or fiber(+) diet (B, D, F) for 14 days. The intestinal sections were incubated with rabbit anti-human pIgR antibodies (bar, 200 μm).

pIgR protein tended to be lower in rats fed the fiber(–) than in those fed fiber(+) diet in the ileum (74% vs. 100%; $p = 0.15$) and the proximal colon (54% vs. 100%; $p = 0.12$). Conversely, there was no significant difference in the pIgR levels between the groups in the jejunum (96% vs. 100%; $p = 0.89$). The amount of pIgR mRNA did not significantly differ between the groups in the proximal and distal colons (Fig. 4).

DISCUSSION

We investigated the effects of oral administration of a fiber-free diet on the secretory immune system in the intestine. For 2 weeks, young rats (4 weeks old) were fed either a fiber(–) diet (cellulose- and starch-free) with only sucrose as the carbohydrate source or a fiber(+) diet formulated to include dietary fiber from beets, which is a naturally occurring complex dietary fiber. The IgA content in 24-hr fecal samples, the IgA content in intestinal tissue, and the amount of pIgR (mRNA and protein) in intestinal tissue were compared between the 2 groups. The IgA content in the 24-hr fecal sample from the fiber(–) diet group decreased to as low as 27% compared with the fiber(+) diet group. This result

suggests that dietary fiber deficiency caused suboptimal conditions in the secretory immune system.

Theoretically, there are at least four explanations for the reduction of fecal IgA in rats fed the fiber(–) diet. First, IgA production by plasma cells in the intestinal lamina propria might be reduced by dietary fiber deficiency. Second, there might be some defects in IgA transport across the intestinal epithelial layer in rats fed the fiber(–) diet. Third, dietary fiber deficiency might reduce bile-derived IgA, because some amount of SIgA in the feces is originally transported from the circulation into the bile in rats [17]. Finally, the stability of SIgA in the intestinal lumen might be decreased by dietary fiber deficiency. In the present study, we tested the former two possibilities. There was no difference in the IgA content in the tissues of the small intestines and colons. These data were consistent with those of previous studies that compared IgA levels in small intestinal tissue extracts of rats fed fiber-free diets (TPN or nutrient-defined diet) and bulk fiber-containing diets [5, 7]. As a previous report presented that the rat intestinal villi contained predominantly IgA plasma cells [18], the IgA content in the tissues of the intestines was related to IgA production by the plasma cells. Accordingly, the decreased IgA
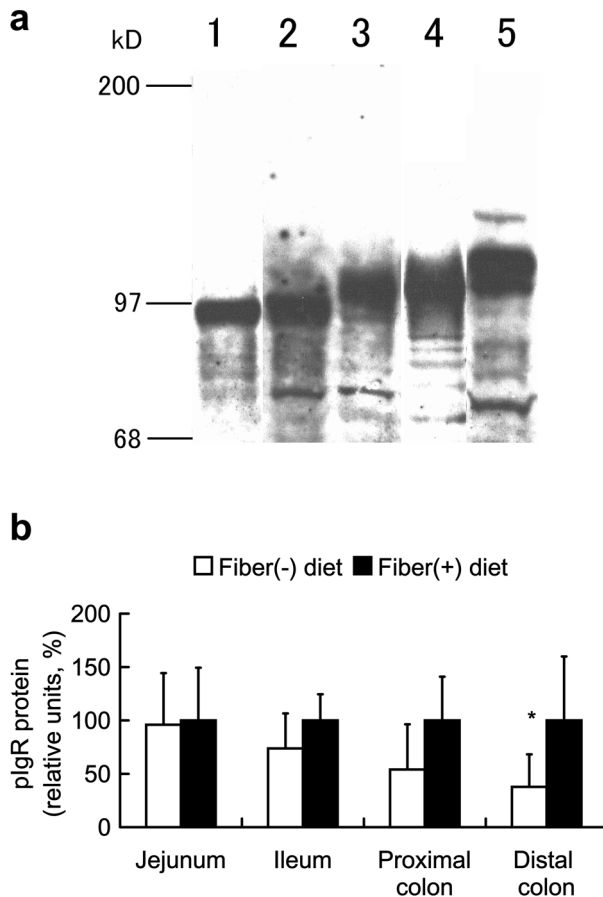
a

b

Fig. 3.   Quantification of pIgR protein in the intestines. Four-week-old rats were administered either a fiber(−) or fiber(+) diet for 14 days. Intestinal plasma membrane samples were prepared from the jejunum, ileum, proximal colon and distal colon. Intestinal pIgR protein levels were examined by Western blot analysis. (a) The representative immunoblots of intestinal pIgR protein in the jejunum (lane 1), ileum (lane 2), proximal colon (lane 3) and distal colon (lane 4) of rats fed the fiber(+) diet are shown. The immunoblot of the liver plasma membrane from a 10-week-old rat as the positive control is also shown (lane 5). (b) The relative quantities of pIgR protein were estimated by densitometric scanning. The values were expressed relative to the average value of the fiber(+) diet group that was normalized to 100. The results are expressed as mean + SD values ($n = 6$). *A significant difference from the fiber(+) diet group ($p < 0.05$) was observed using the Student's t-test.

content in the feces of the fiber(−) diet group was not caused by a decrease in IgA production by the plasma cells in the lamina propria of the intestinal mucous membrane. Further, immunohistochemical and immunoblotting analyses of the small intestine and colon confirmed that the fiber(−) diet group showed a significant decrease in pIgR protein expression in the distal colon compared with the fiber(+) diet group.
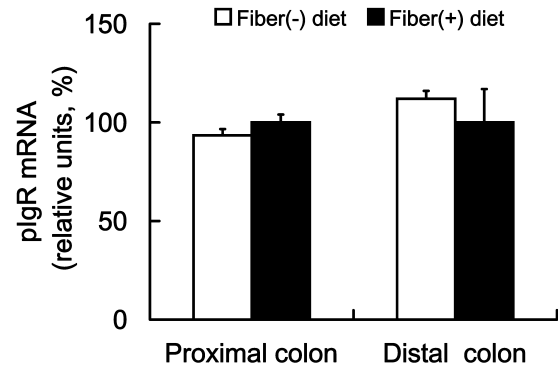

Fig. 4.   Quantification of pIgR mRNA in the intestines. Four-week-old rats were administered either a fiber(−) or fiber(+) diet for 14 days. Total RNA was prepared from the proximal and distal colons. Intestinal pIgR mRNA levels were examined by Northern blot analysis. The relative quantities of pIgR mRNA were estimated by densitometric scanning. The values are expressed relative to the average value of the fiber(+) diet group normalized to 100. The results are expressed as mean + SD values ($n = 6$).

Because pIgR plays a central role in the transepithelial migration of IgA in the intestine [2], the data suggest that dietary fiber deficiency reduces the secretion of IgA in the colon, due to reduced expression of pIgR. In addition, because pIgR contributes to the stability of SIgA by protecting proteolytic degradation in the intestinal lumen [2], the reduced expression of pIgR may also lead to increased degradation of SIgA in the intestinal lumen. Therefore, we believe that the reduced fecal IgA in rats fed the fiber(−) diet is, at least in part, attributable to the decrease in pIgR expression in the colon.

The intestinal secretory immune system is generated by a cooperation between 2 distinct cell-types: lymphatic plasma cells that produce pIgA and nonlymphatic epithelial cells that express pIgR. Although the administration of a fiber(−) diet reduced the expression of pIgR in the large intestines of young rats, the IgA content in the intestinal tissue did not differ between the fiber(−) diet and fiber(+) diet groups. This suggests that a fiber-free diet's influence on the secretory immune system may be triggered by its influence on the intestinal epithelial cells rather than on plasma cells. Sano et al. [7] reported a similar finding in their study in which they compared the oral administration of TPN (a fiber-free diet) with the Chow diet (a fiber containing diet). However, Sano et al. found that pIgR expression decreased in the small intestine, while we found that it is not changed. While the reason for this difference is not

clear, the experimental design of the present study was different from that used by Sano et al. The diet used by Sano et al. differed not only in fiber content but also in nutritive composition. For example, the nitrogen source in TPN was an amino acid, whereas in the Chow diet it was a protein. Furthermore, Sano et al. used a different experimental model (mouse) than that (rat) used in our study.

We previously reported that administering a fructooligosaccharide, which is a type of prebiotic, produced an increase not only in pIgR expression in the intestines of infant mice but also an increase in the IgA content in their intestinal tissues, the IgA secretion rate, and the number of B220+IgA+ B cells in Peyer's patches [13]. Ito et al. [19, 20] similarly reported that the administration of inulin-type fructans increases IgA levels in the contents of the cecum of rats, as well as the number of IgA-producing plasma cells in the intestinal tissue. In the present study, young rats fed a fiber-free diet showed reduced pIgR expression in the intestine, but there was no difference in the IgA content in the intestinal tissues, as compared with rats fed the fiber(+) diet. It is unclear why dietary indigestible carbohydrates, such as fructooligosaccharide and inulin-type fructans, would have different effects on the secretory immune system of the intestine than dietary fiber. However, it is possible that they have different effects on the gut flora. That is, fructo-oligosaccharide and inulin-type fructans increase the number of bifidobacteria and lactobacilli in the intestine [20, 21], while dietary fiber from beets, which was used as the source of dietary fiber in the fiber(+) diet in the present study, does not affect the number of bifidobacteria, lactobacilli, or obligate anaerobic bacteria in the intestine [22]. If we consider that bifidobacteria increase the amount of IgA in the intestines of mice [23, 24], then diet-induced changes in the intestinal microflora may be important regulators of the IgA content in the intestinal tissues.

Sullivan et al. [25] found that a protein- and energy-deficient diet decreased pIgR production in the small intestines of rats after weaning (3 to 11 weeks old), indicating that protein intake is critical to the regulation of the secretory immune system during growth. In our study, which used young rats (4 weeks old), both the fiber(−) and fiber(+) diets had sufficient protein to support the rats' growth, and we did not observe any reduction in body weight, as was observed with protein- and energy-deficient diets. Since the fiber(−) diet significantly reduced pIgR expression in the large intestine compared with the fiber(+) diet, we suggest that in young animals, fiber intake is an additional factor important for the regulation of the secretory immune system.

Why does a fiber-free diet reduce pIgR expression in intestinal epithelial cells? An in vitro study reported that butyric acid, a short-chain fatty acid, directly increases pIgR production in colon cancer cells [26]. When orally administered to rats, dietary fiber from beets increases the concentration of short-chain fatty acids, such as acetic acid, propionic acid, and butyric acid [27, 28], in the cecum. Therefore, an in vivo study to assess the possibility that butyric acid or other fatty acids in the intestine regulate pIgR expression needs to be performed.

In the present study, the lack of differential pIgR mRNA expression in the colonic tissue between the fiber(−) and fiber(+) diet groups suggested that the fiber-free diet decreased expression of pIgR protein due to a posttranscriptional mechanism. Recently, it has been indicated that the expression of pIgR is regulated by a posttranscriptional mechanism [29]. Accordingly, a future study to examine how posttranscriptional regulation influences the decreased expression of pIgR in a fiber(−) diet needs to be performed.

Our results suggest that dietary fiber deficiency decreases pIgR protein expression in the intestine and, as a result, leads to suboptimal defenses against infection by intestinal SIgA. The decreased expression of pIgR caused by a fiber-free diet may be attributable to a posttranscriptional mechanism; therefore, further studies are required to clarify the mechanism of this influence.

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