Secretion and Excretion of Immunoglobulin A to Cecum and Feces Differ with Type of Indigestible Saccharides

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Summary The study was conducted to elucidate the effects of orally administered indigestible saccharides (IDS) on immunoresponses of the intestinal tracts, especially secretion and excretion of immunoglobulin A (IgA). Male 4-week-old Sprague-Dawley rats were fed diets containing several kinds of IDS (cellulose, corn husk, glucomannan, curdlan and lactulose) at 5% for three weeks. The results indicated that the proportion of IgA-presenting lymphocytes in the cecal mucosa of the tested IDS groups increased significantly or tended to increase compared with that of the cellulose group. No significant differences among the experimental groups were observed in the CD4+- and CD8+-presenting lymphocytes and the CD4+/CD8+ ratios in the small intestine, cecum and mesenteric lymph nodes. IgA amounts in the cecal contents increased significantly in the glucomannan and curdlan groups as compared with that in the cellulose group. The inconsistent results were observed in the cecal IgA amounts of the lactulose group. Although IgA excretion into feces increased periodically in the cellulose, hardly any changes were observed in the glucomannan and curdlan groups. These results revealed that IgA secretion from cecal mucosa to contents was promoted, and its excretion to feces was decreased by the oral administration of highly fermentable IDS, respectively, while non- or low-fermentable IDS functioned adversely to IgA responses in the intestinal tract. It is suggested that the response of IgA in the intestinal immune system differs with the type of IDS ingested.

Key Words indigestible saccharides, IgA secretion, intestinal immunity, IgA-presenting lymphocytes, T lymphocytes

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Past research on the relation between immunity and dietary fibers (DF) mainly dealt with in vitro studies or in vivo those by intraperitoneal injections (1-4), and only a very few dealt with the effects of oral administration, leaving many questions unanswered. Especially, as little interest has been shown so far regarding the effects of DF on the intestinal immune system, there is practically no information in this field.

Recently, Lim et al (5) reported that the oral administration of pectin, water-soluble DF, increased the proportion of CD4+ -presenting lymphocytes and the CD4+/CD8+ ratio in T lymphocytes, elevated the concentration of IgM, IgG and IgA and decreased that of IgE in mesenteric lymph nodes (MLN), suggesting its involvement with intestinal tract immunity. Kudoh et al (6) observed that the oral administration of IDS increased the proportion of B lymphocytes in the small intestine and cecal mucosa, and suggested that lactic acid or lactic acid-producing bacterium in the cecum might be responsible.

The intestinal tract has gut-associated lymphoid tissues (GALT) which are responsible for protecting the host from invasion of bacterium and viruses through local immunization by IgA secreted from the mucosa (7, 8). GALT are also involved in allergies induced by antigens from foods. The increased proportion of B lymphocytes in the mucosa of the intestinal tract induced by the oral administration of IDS is presumed to promote the secretion of IgA into the intestinal tract, which in turn activates local immunity in the host. However, it has not been clarified yet how dietary IDS influences the secretion of IgA from GALT into the intestinal tract.

We therefore studied the effects of oral administration of IDS on secretion and excretion responses of IgA and also T lymphocyte responses in the intestinal mucosa and MLN.

MATERIALS AND METHODS

Animals, diets and rearing methods. Male 4-week-old Sprague-Dawley rats (Tokyo Experimental Animal Science Co., Tokyo, Japan) were used for the two experiments. IDS used for Experiment 1 were cellulose (CP, Oriental Yeast Co., Tokyo, Japan), glucomannan (GM, Shimizu Kagaku Co., Hiroshima, Japan), corn husk (celfur: CF, Nihon Shokuhin Kako Co., Tokyo, Japan) and lactulose (LL, Nikken Kagaku Co., Tokyo, Japan). CP was used as a control. For Experiment 2, CF was replaced with curdlan (CD, Takeda Chemical Industries, Co., Osaka, Japan). For both experiments, after one week of preliminary rearing on the basal diet (AIN-76), rats were divided into four groups (Experiments 1 and 2) of seven per group and given diets containing IDS as mentioned above. The composition of the diet (g/100 g diet) was as follows; casein 20, corn oil 5.0, DL-methionine 0.3, corn starch 15, vitamin mixture (AIN-76) 1.0, mineral mixture (AIN-76) 3.5, choline chloride 0.2, IDS 5.0 and sucrose up to 100. The diets and water were given ad libitum. As LL induces diarrhea or soft stools, rats were acclimatized to a diet containing IDS at 2% in the first week, and then given the diet containing IDS at
5% for the next two weeks in Experiment 1, and that for three weeks in Experiment 2. Feces were collected during the last three d of each week. Care and use of the rats in the present study were followed by the guidelines of governmental legislation in Japan (1980).

Separation of lymphocytes from intestinal mucosa and MLN. Rats were sacrificed by withdrawing blood from the heart under pentobarbital anesthesia. The intestinal tract (small intestine and cecum) was extirpated, washed with cold phosphate-buffered saline (PBS, pH 7.2), and cut open to separate lymphocytes using collagenase C-6885 (Sigma Chemical Co., St. Louis, MO, USA) (9). Lymphocytes in MLN were prepared by the method of Jürgen et al (10) and used for separation of splenic cells.

Analysis of lymphocytes by flowcytometer. Lymphocyte classes of the obtained cell groups were gated, and the proportions of IgA (MARA-1-F; Experimental Immunology Unit, Brussels, Belgium), CD4 (MCA-55F, Serotec, Oxford, England) and CD8 (MCA-48P, Serotec, Oxford, England)-presenting lymphocytes in the small intestinal and cecal mucosa were analyzed by a flowcytometer (Coulter Epics Elite, Coulter Electronics, Miami, USA) using monoclonal antibodies. The proportions of CD4- and CD8-presenting lymphocytes in MLN were similarly measured using a flowcytometer.

Analysis of IgA in serum, cecal contents and feces. The cecal contents were diluted with PBS containing 0.5% Tween 20 and 1% bovine serum albumin (BSA), and centrifuged to obtain supernatant at 3,500 rpm for 10 min. Feces were freeze-dried, pulverized and diluted with PBS containing 0.5% Tween 20 and 1% BSA, and then centrifuged to obtain supernatant in the same manner as cecal contents. The amounts of IgA in serum, the cecal contents and feces were measured by the enzyme-linked immunosorbent assay (ELISA) method (11).

Statistical analysis. The intergroup significant difference of the means was tested by one-way ANOVA using SPSS, and then for parametric analysis by Duncan’s multiple range test. In the case of heteroscedasticity, Kruskal-Wallis test was performed for non-parametric analysis.

RESULTS

Body weight gain, weight of digestive organs and pH of cecal contents

Table 1 shows the results of Experiment 1. The body weight gain of the GM group was significantly less than the other three groups. No intergroup significant difference was observed in the food intake. The weight of the small intestine, cecum and cecal contents in the GM group increased significantly as compared with that in the CP group. The pH value of the cecal contents in the GM and LL groups was significantly lower than that in the CP group.
Table 1. Body weight gain, food intake, food efficiency, weight of digestive organs and pH of cecal contents.1

<table>
<thead>
<tr>
<th>Item</th>
<th>CP2 (±SE)</th>
<th>GM2 (±SE)</th>
<th>CF2 (±SE)</th>
<th>LL2 (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g)</td>
<td>176 ± 4a</td>
<td>160 ± 3b</td>
<td>180 ± 5a</td>
<td>175 ± 6a</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>475 ± 19</td>
<td>411 ± 21</td>
<td>453 ± 19</td>
<td>437 ± 21</td>
</tr>
<tr>
<td>Food efficiency</td>
<td>0.38 ± 0.01</td>
<td>0.40 ± 0.01</td>
<td>0.40 ± 0.01</td>
<td>0.40 ± 0.01</td>
</tr>
<tr>
<td>Small intestine (g)</td>
<td>7.8 ± 0.3a</td>
<td>11.4 ± 0.6b</td>
<td>8.4 ± 0.4a</td>
<td>8.5 ± 0.5a</td>
</tr>
<tr>
<td>Cecum (g)</td>
<td>0.4 ± 0.0a</td>
<td>1.3 ± 0.1b</td>
<td>0.6 ± 0.0bc</td>
<td>0.8 ± 0.1bc</td>
</tr>
<tr>
<td>Cecal contents (g)</td>
<td>1.8 ± 0.1a</td>
<td>4.5 ± 0.9b</td>
<td>2.2 ± 0.2a</td>
<td>2.1 ± 0.1a</td>
</tr>
<tr>
<td>pH of cecal contents</td>
<td>7.73 ± 0.06a</td>
<td>6.24 ± 0.19b</td>
<td>7.53 ± 0.16bc</td>
<td>7.07 ± 0.15bc</td>
</tr>
<tr>
<td>Colon (g)</td>
<td>0.4 ± 0.0a</td>
<td>1.3 ± 0.1b</td>
<td>0.6 ± 0.0bc</td>
<td>0.8 ± 0.1bc</td>
</tr>
</tbody>
</table>

1 Values are means ± SE (n=6 or 7). Values with different superscript letters are significantly different (p<0.05).
2 CP, cellulose; GM, glucomannan; CF, celfur; LL, lactulose.

Fig. 1. Proportions of IgA-, CD4+- and CD8+-presenting lymphocytes in the intestinal mucosa and mesenteric lymph nodes (MLN). □: small intestine, ■: cecum, □: MLN. Abbreviations: CP, cellulose; GM, glucomannan; CF, celfur; LL, lactulose. Values are means ± SE (n=6 or 7). Values with different superscript letters in the same kind of column are significantly different (p<0.05).
Proportions of IgA-, CD4\(^+\)- and CD8\(^+\)-presenting lymphocytes in intestinal mucosa and MLN

Figure 1 shows the results of Experiment 1. The proportion of IgA-presenting lymphocytes in the mucosa of small intestine was not significantly different among the groups, but that in the cecal mucosa of the CF and LL groups increased significantly or tended to increase compared with that of the CP group. The proportions of CD4\(^+\)- and CD8\(^+\)-presenting lymphocytes and the ratios of CD4\(^+\)/CD8\(^+\) in the intestinal mucosa, cecal mucosa and MLN of the tested IDS groups did not show any significant difference as compared with those of the CP group.

Concentration of IgA in serum

Figure 2 shows the results of Experiment 1. No significant intergroup differences were observed in the serum IgA concentration. But those of the tested IDS groups showed decreasing tendencies as compared with that of the CP group. Similar results were obtained in Experiment 2 (data not shown).

Amounts of IgA in the cecal contents and feces

Figure 3 shows the results of Experiments 1 and 2. In Experiment 1, IgA amounts per entire cecal contents increased significantly in the GM group and tended to increase in the CF and the LL groups as compared with that in the CP group, respectively. In Experiment 2, IgA amounts per entire cecal contents in the CD and GM groups increased significantly as compared with that in the CP group.

Figure 4 shows the fecal output and IgA excretion in feces per d in Experiment 2. Fecal weight and IgA amounts in the feces of the tested IDS groups showed significant decreases or decreasing tendencies at weeks 1, 2 and 3 as compared with those of the corresponding weeks in the CP group, respectively. In the CP group, the fecal output and fecal IgA excretion per d at week 3 increased significantly \((p<0.05)\) as compared with those at weeks 1 and 2, but those in the tested IDS
DISCUSSION

Authors (6) previously reported that the oral administration of certain IDS increased the proportion of B lymphocytes in the mucosa of the small intestine and cecum as compared with the control (cellulose), and observed that IDS such as DF affected the immunity of the intestinal tract. In this study, we again observed that the oral administration of IDS increased the proportion of IgA-presenting lymphocytes in the cecal mucosa.

We therefore measured the amounts of IgA secreted into the cecum and found in Experiment 1 that GM and LL, which are highly fermentable IDS, increased secretion as compared with CP, which is not fermentable. The results in Experiment 2 revealed significant increases of IgA secretion into the cecum in the groups fed fermentable CD and GM as compared with the CP-fed group. It was assumed that
the enhancement of IgA secretion from the cecal mucosa might be caused by the increase of various antigens such as intestinal flora, cell wall substances or decomposed and fermented products of IDS by bacterium in the cecum. Although no significant increase was observed in the proportion of IgA-presenting lymphocytes in the GM group, IgA in the cecal contents increased significantly. This might be due to the increase of the total number of IgA-presenting lymphocytes by the increased weight of the cecum in the GM group.

On the other hand, IgA excretion into the feces of rats fed the tested IDS decreased significantly or showed a decreasing tendency as compared with the CP group at weeks 1, 2 and 3, respectively. The IgA amounts in the feces increased with the passage of rearing days in the CP group, but showed no change or decreasing tendency in the tested IDS groups. This might be due to the fact that the daily fecal output of the CP group increased with time, thereby increasing the IgA amounts, whereas the daily fecal output of the CD, GM and LL groups hardly changed. It is known that feces contain various bile acids and their metabolites, and the amounts are influenced by the quality and quantity of DF ingested (12, 13). It is also known that bile contains IgA (14). The oral administration of some kinds of IDS may possibly alter the quantity of bile and the composition of bile acids, and thus affect IgA excretion into the feces.

Serum IgA concentration showed a decreasing tendency in the tested IDS groups. This slight decrease of serum IgA concentration might be due to a lesser burden on the whole body immunity by the activation of intestinal immune responses. Reporting on the IgA concentration in serum, Lim et al (5) noted that the concentration fluctuates depending on the type of DF given and there is no uniform tendency.

On the other hand, the proportions of CD4+ and CD8+-presenting lymphocytes and the CD4+/CD8+ ratios in the mucosa of the small intestine, cecum and MLN were not significantly different among all the groups. Thus, the oral administration of IDS did not markedly affect T lymphocytes in the mucosa of the intestinal tract. Lim et al (5) reported that pectin administration significantly increased the ratio of CD4+/CD8+ of T lymphocytes in MLN, but other DF did not. The response therefore varies depending on the type of DF. It is known that interleukin (IL) 4 and IL-5 secreted from T lymphocytes mediate the differentiation and proliferation of B lymphocytes (15). The CD4+ cells consist of helper T (Th) 1 cells and Th2 cells. Th1 cells are known to produce interferon (IFN)-γ, and Th2 cells IL-4, IL-5 and IL-10 (16, 17). There is a deep relation between Th1 and Th2 cells as IFN-γ restrains the proliferation of Th2 and IL-10 restrains lymphokine production by Th1. Even if there were no remarkable differences observed in the proportions of CD4+ and CD8+ cells, it is possible that changes may take place in the proportions of Th1 and Th2 cells in the CD4+ cells or the production of interleukin per se. Takeuchi et al (18) and Takimoto et al (19) reported on changes in the composition of the T cells of intraepithelial lymphocytes in the intestinal tract accompanying the aging of mice and rats. According to them, changes in the
intestinal flora due to aging affect the composition of T lymphocytes.

IDS reaches the lower part of the intestinal tract without being decomposed by digestive enzymes, and is decomposed and fermented by intestinal bacteria residing there to produce organic acids such as short-chain fatty acids and lactic acid. The kinds and amounts of organic acids vary depending on the type of IDS (20–22).

Among short-chain fatty acids, butyric acid is known to promote the proliferation of epithelial cells of the intestinal tract. Eftimiadi et al. (23) reported that propionic acid, butyric acid and isobutyric acid affect the blastogenesis of T cells in vitro, and that butyric acid, in particular, affects the production of IL-1β. It is also conceivable that changes in intestinal flora and amounts of short-chain fatty acids caused by IDS ingestion may affect the differentiation and proliferation of T cells or the production of cytokines in the immune system of the intestinal tract.

We, however, did not observe responses of T lymphocytes when the proportions of CD4+ and CD8+ were used as an indicator. Anyway, our results showed that IgA-presenting lymphocytes in the cecal mucosa, IgA secretion to cecal contents and IgA excretion to the feces are affected differently depending on the type of IDS administered orally.

This finding is very interesting and meaningful in the aspect of protection of the host from invasion of various antigens. It is assumed that some fermentable IDS act to modify the responses of B lymphocytes in the immune system of the intestinal tract. However, further study to solve the mechanisms of increase of IgA secretion is warranted in regards to the problems of T-lymphocyte responses including cytokine production and the increase of antigens.

REFERENCES


