Effect of Dietary Pectin on the Production of Immunoglobulins and Cytokines by Mesenteric Lymph Node Lymphocytes in Mouse Colitis Induced with Dextran Sulfate Sodium

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The present study explores the dietary effect of pectin on the MLN lymphocyte functions of mice with dextran sulfate sodium (DS)-induced colitis. We found that the immunoglobulin (Ig)A level in mesenteric lymph node (MLN) lymphocytes was high, while the IgE level was lower, in mice fed with pectin than in those fed with cellulose. Interestingly, the fecal IgA concentration of the pectin-fed mice was significantly higher than that of the cellulose-fed mice. The concentrations of interferon-γ and interleukin (IL)-2 treated with concanavalin A (ConA) were significantly higher in the pectin-fed group than in the cellulose-fed group. Although dietary pectin did not affect the IL-4 and IL-10 levels, the activation-induced IL-4 and IL-10 secretion was lower in MLN cells of the pectin-fed mice than of the cellulose-fed mice following DS-induced colitis. Based on these findings, we propose that the effect of dietary pectin on mice with DS-induced colitis is mediated by the manipulation of Th1 cells. Furthermore, the inhibitory effect of IL-4 and IL-10 by dietary pectin may play an important role in promoting a change in Th1/Th2 balance toward Th1-dominant immunity.

Key words: inflammatory bowel disease; dietary pectin; mesenteric lymph node; dextran sodium sulfate; fecal IgA

Inflammatory bowel disease (IBD), including Crohn's disease (CD), and ulcerative colitis (UC) are chronic, relapsing, and remitting conditions of unknown origin. IBD exhibits various characteristics of immunological inflammation, affecting at least one in 1,000 people in Western countries.1,2 An increasing amount of evidence shows that the immune system plays a critical role in the development and perpetuation of UC and CD. These previous studies on humans for the pathogenesis of IBD1,2,4 have implicated an impaired mucosal barrier function, marked innate immunity, the production of cytokines, and the activation of CD4+ T cells. Proinflammatory cytokines have been shown to be involved in the initiation and perpetuation of IBD.5,6

Recent studies have shown that the production of such cytokines as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and IL-8 at the sites of inflammation were markedly enhanced in patients with UC and in those with CD.7-9 The results of therapeutic trials on cytokine manipulation in such patients further support the idea that cytokines are important in the pathophysiology of these diseases.4,10,11

There has been increasing recent interest in both the physiological and pharmacological effects of dietary fiber.12,13 We have previously reported that water-soluble dietary fiber might have an immunoregulatory function in the intestinal immune system.14-16 In addition, dietary pectin might regulate immunoglobulin (Ig) production by mesenteric lymph node (MLN) lymphocytes, and it was suggested that pectin exerted anti-allergic activity.14,17 Our previous results have shown that the effect of dietary pectin was exerted through Th1 cells.14,15

IFN-γ production and IL-2 receptor expression are both increased with dietary pectin in the MLN.14,15 Th1 cytokines as IFN-γ and IL-2 can oppose the Th2 function and/or its development, and thereby inhibit the production of IL-4 and IL-10. In addition, dietary pectin inhibits the synthesis of the pro-inflammatory cytokine, TNF-α. We therefore examined whether dietary pectin would have an effect on Th1 in improving the immunological expression of colitis by showing a decrease in the production of intestinal inflammatory parameters such as IL-4 and IL-10 by Th2 cells.

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Abbreviations: ELISA, Enzyme-linked immunosorbent assay; DS, dextran sulfate sodium; ConA, concanavalin A; IBD, inflammatory bowel disease; MLN, mesenteric lymph node; IFN, interferon; IL, interleukin; FBS, fetal bovine serum
Many studies have shown that the water-soluble dietary fiber, pectin, is an important precursor of those substrates necessary for the intestinal structure and function. However, few studies have examined the effects of pectin by utilizing a model involving chemotherapeutic-induced colitis. The purpose of this current study is to evaluate the intestinal immunoregulatory effects of pectin on dextran sulfate sodium (DS)-induced colitis in mice. The oral administration of DS to mice induces colitis resembling human UC. This model shows signs corresponding well to the clinical signs of IBD in humans and can serve as a reliable model for studies of this disease. In the present study, we focused on the response of MLN lymphocytes in mice after DS-induced colitis, and found a significantly greater effect of dietary pectin on Ig and cytokine production compared than that cellulose.

Materials and Methods

**Materials.** Pectin (from apple) was purchased from Sigma (St. Louis, MO, U.S.A.) as were concanavalin A (ConA) and dextran sulfate sodium (DS). ConA and DS were dissolved in phosphate-buffered saline (PBS at pH 7.4) or water and used for the cell culture experiments. Monoclonal antibodies and cytokines were purchased from ID Labs (Ontario, Canada), the antibodies for IgA determination were purchased from Zymed Laboratories (San Francisco, CA, U.S.A.), and the antibodies for IgE determination were purchased from Biosource International (Comarillo, CA, U.S.A.). The enzyme-linked immunosorbent assay (ELISA) for mice IgE and IgA used 0.05% Tween 20 in PBS (TPBS) for rinsing and Block Ace (Dainihon Pharmaceutical Co., Osaka, Japan) for blocking and dilution of the antibodies as described previously. All other reagent-grade chemicals were purchased from Sigma (St. Louis, MO, U.S.A.).

**Induction of colitis.** Colitis was induced by feeding drinking water supplemented with 5% DS. This model has been described in detail previously. The cellulose mice were treated in a similar manner with tap water under a controlled temperatures (25 ± 2°C). They were given the AIN-76 diet and drinking water without DS for 5 days. After 5 days, these mice were given the AIN-76 diet without DS for 5 days, and then pectin was added to the diet at the 5% level in place of cellulose in the AIN 76 diet for 2 weeks. The DS + pectin group was given the AIN-76 diet and drinking water without DS for 5 days, and then pectin was added to the diet at the 5% level in place of cellulose in the AIN 76 diet for 2 weeks.

**Preparation of mesenteric lymph node lymphocytes.** MLN was excised from the Balb/c mice, and lymphocytes were squeezed out into an the RPMI 1640 medium (Invitrogen Corporation, Grand Island, NY, U.S.A.). After incubating the cells at 37°C for 30 min to remove fibroblasts, 5 ml of the cell suspension was layered on 4 ml of Lympholyte-mice (Cedarlane, Hornby, Canada), before being centrifuged at 1,500 × g for 30 min. The lymphocyte band at the interface was recovered, and the cells were rinsed three times with the RPMI 1640 medium. The lymphocytes were cultured in 10% fetal bovine serum (FBS; Invitrogen Corporation, Grand Island, NY, U.S.A)/RPMI 1640 medium, and the IgE and IgA contents of the culture supernatant were measured by ELISA. The cell viability was measured by trypsin blue staining, the viability by this preparation being more than 95% of the total cells.

**Isolation of mesenteric lymph node T-lymphocyte subsets.** To MLN lymphocytes suspended at 1 × 10^6 cells/100 μl of 10% FBS/PBS was added 5 μl of either CD4-FITC or CD8-PE monoclonal antibodies (Santa Cruz Biotechnology, Inc, USA), and incubated at 4°C for 30 min. The lymphocytes were rinsed three times with PBS containing 10% FBS and centrifuged at 300 × g for 5 min. The stained lymphocytes were fixed by 2% paraformaldehyde and count- by EPICS Altra™ flow cytometry (Beckman Coulter, U.S.A). Each analysis, including that of negative control samples, was based on at least 10^4 events after the dead cells and residual erythrocytes had been eliminated by gating on the basis of the forward angle light scatter.
**Fecal IgA measurement.** Fecal pellets from each mouse in each group were collected from 0 to 14 days, and stored at −80°C. These samples were collected and prepared as described by Fukushima et al. In brief, fecal pellets were collected from each mouse, placed in individual microcentrifuge tubes and suspended in a protease inhibitor (0.1 g/ml, containing of 0.1 mg/ml trypsin inhibitor, 50 mM EDTA and 1 mM phenylmethanesulfonyl fluoride) for 1 hr at 4°C. The pellets were homogenized with a vortex mixes and centrifuged at 15,000×g for 10 min. The resulting supernatant fraction were collected and kept at −80°C prior to the IgA antibody measurements.

**Measurement of cytokines.** The supernatant from each 48 h ConA-activated MLN lymphocyte culture was obtained. Cytokines (IFN-γ, IL-2, TNF-α, IL-4, and IL-10) were measured by ELISA by using cytokine-specific capture and detection monoclonal antibodies as previously described. Statistically, Differences between the means of the individual groups were assessed by a one-way analysis of variance with Duncan's new multiple-range test (SPSS version 7.5, SPSS Institute, Chicago, IL, U.S.A.). Differences of P < 0.05 are considered to be significant.

**Results**

**Growth variables**

The food intake and body weight are shown in Table 1. The body weight of each mouse in the dextran sodium sulfate (DS) + cellulose and DS + Pectin groups was significantly lower than that of the mice fed on cellulose or pectin alone. The DS + cellulose group had a lower food intake than the other groups. The difference between the cellulose group and pectin group was significant, but was not significantly different for the DS + cellulose and DS + pectin groups.

**Fecal IgA concentration**

After feeding the mice for 5 days to achieve DS-induced acute colitis, the concentration of IgA in the fecal extract was measured over a disease period of 14 days. The results are shown in Fig. 1. The cellulose- and pectin-fed groups showed a similar response pattern in which the IgA level remained unchanged until 5 days. From day 4 to day 11, the IgA level in the pectin group was higher than that in the cellulose group. Thereafter, the cellulose and pectin groups showed a similar response pattern in which the IgA level remained unchanged until day 14. In the DS group, the IgA level decreased for 9 days after DS-induced colitis. The peak IgA level of the cellulose group was between that of the pectin and DS groups. More interestingly, the IgA level of mice in the pectin group reached a maximum level from 4 to 7 days, and then decreased rapidly. From day 9 to day 14, the pectin and DS + pectin groups showed a similar response pattern in which the IgA level remained change.

**Mesenteric lymph node (MLN) Ig concentration**

In the absence of ConA, the IgA concentration after 48 h of incubation of the MLN lymphocytes was higher in the mice fed on pectin than in those fed on cellulose (Fig. 2). IgA was significantly lower in the DS group than in cellulose, pectin, and DS + pectin groups. The ConA treatment increased the concentration of IgA in cells isolated from both the cellulose- and pectin-fed rats, with the value markedly higher in the latter group. The IgA concentration in MLN lymphocytes from the DS + pectin group was higher than that in cells from the DS group. In contrast, the IgE concentration in MLN lymphocytes from the DS group was higher than that in the mice fed on DS + Pectin when ConA was absent. When the cells were cultured with ConA, a similar response pattern was observed.

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**Table 1. Food Intake and Body Weight of Mice Fed on the Experimental Diets for 2 Weeks**

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Initial body weight (g)</th>
<th>Final weight (g)</th>
<th>Food intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>18.4 ± 0.1a</td>
<td>21.2 ± 0.3a</td>
<td>12.6 ± 0.1a</td>
</tr>
<tr>
<td>DS + Cellulose</td>
<td>18.1 ± 0.2a</td>
<td>17.3 ± 0.1b</td>
<td>10.2 ± 0.9b</td>
</tr>
<tr>
<td>Pectin</td>
<td>18.2 ± 0.2a</td>
<td>19.3 ± 0.2c</td>
<td>12.3 ± 0.1a</td>
</tr>
<tr>
<td>DS + Pectin</td>
<td>18.2 ± 0.1a</td>
<td>17.7 ± 0.1b</td>
<td>10.9 ± 0.6ab</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE of 6 mice. Values without the same superscript letter are significantly different at *p < 0.05.

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**Fig. 1.** IgA Content in Fecal Extracts Prepared from Normal and DS-Induced Colitis Mice Fed on Cellulose or Pectin.

Open diamonds: mice fed on the AIN 76 diet for 2 weeks. Open squares: mice given pectin in place of cellulose in the AIN 76 diet. Black triangles: mice given the AIN-76 diet for 2 weeks, after 5 days of dextran sodium sulfate. Black circles: mice given the AIN 76 diet containing pectin for 2 weeks, after 5 days of dextran sodium sulfate.
MLN lymphocyte T cell population

The proportion of CD4+ and CD8+ T cells in MLN lymphocytes was measured in relation to the changes in Ig concentration. (Table 2) We found a significant increase in the relative population of CD4+ T cells in the DS-induced colitis group compared to those fed cellulose and pectin. On the other hand, the proportion of CD8+ T cells was higher in the cellulose group than in the pectin group. However, the proportion of CD8+ T cells in cellulose and DS + Pectin groups remained. Consequently, the ratio of CD4+/CD8+ cells was lower in mice fed cellulose than the other groups.

Cytokine concentrations in MLN lymphocytes

Various types of lymphokines specifically regulate Ig production by class. When lymphocytes were cultivated for 48 h without ConA, the concentration of IL-2 was generally below the detection limit (Table 3), although the cells from mice fed on dietary pectin alone had a detectable concentration of IFN-γ, while TNF-α was detected at a low concentration in the DS + cellulose group. In the presence of ConA, the levels of IFN-γ and IL-2 were higher in all the cells from the pectin-fed groups, although the effect on TNF-α was less marked.

DS-induced colitis was accompanied by a disturbance in the IL-4 and IL-10 levels (Table 4). In the absence of ConA, all groups had a detectable concentration of IL-10, while IL-4 was below the detection limit. Activation with ConA significantly enhanced IL-4 and IL-10 production in the cellulose-fed DS-induced colitis group. IL-4 and IL-10 concentrations in the pectin-fed group were no different from those of the cellulose-fed mice. Interestingly, the mice fed...
Table 4. Activation-induced Th2 Cytokine Secretion of MLN Lymphocytes in Normal and DS-Induced Colitis Mice Fed on Celulose or Pectin

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>ConA IL-4 (ng/l)</th>
<th>IL-10 (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>– nd</td>
<td>29 ± 2 ²</td>
</tr>
<tr>
<td>DS + Cellulose</td>
<td>– nd</td>
<td>50 ± 2 ²</td>
</tr>
<tr>
<td>Pectin</td>
<td>– nd</td>
<td>31 ± 1 ²</td>
</tr>
<tr>
<td>DS + Pectin</td>
<td>– nd</td>
<td>38 ± 2 ²</td>
</tr>
<tr>
<td>Cellulose</td>
<td>+ 65 ± 4 ²</td>
<td>68 ± 2 ²</td>
</tr>
<tr>
<td>DS + Cellulose</td>
<td>+ 107 ± 4 ²</td>
<td>94 ± 2 ²</td>
</tr>
<tr>
<td>Pectin</td>
<td>+ 63 ± 4 ²</td>
<td>69 ± 2 ²</td>
</tr>
<tr>
<td>DS + Pectin</td>
<td>+ 47 ± 4 ²</td>
<td>56 ± 3 ²</td>
</tr>
</tbody>
</table>

Data are means ± SE of five mice. a–d Values without the same superscript letter are significantly different at p < 0.05. nd; not detected.

on pectin in the DS-induced colitis group showed lower production of IL-4 and IL-10 than the mice in the other three groups.

Discussion

The underlying mechanism for the effect of dietary fiber on inflammatory bowel disease and the immunological function are not fully understood, although dietary fiber has been reported to influence the immune function through a change in the intestinal microflora. Several studies have shown that the immunoregulatory effect of dietary pectin was apparent when animals were fed for 2 weeks, so we chose this feeding period in the present study.

We developed DS-induced colitis in mice which produced an experimental condition resembling human UC. We examined in the DS-induced colitis mice whether dietary pectin would have a significant effect on Ig and cytokine production in comparison with the effect of cellulose. The type of dietary fiber can influence the composition of short-chain fatty acids (SCFA) produced in the gut. Soluble-fiber pectin is fermented to acetic acid (approximately 80% of total SCFA) and a minimal amount of butyric acid. In addition, dietary SCFA has been shown to increase the T cell number in the gastrointestinal system, suggesting a possible mechanism for the regulatory influence of dietary fiber.

Pectin increases the water-binding capacity of feces, thereby increasing the fecal weight and volume and altering the consistency of the stool. An interesting finding is that pectin is also an important antioxidant, offering mucosal protection against the three main causal agents of oxidative damage: peroxyl, superoxide, and hydroxyl radicals. These effects might explain why pectin is known to stimulate the gut-associated lymphoid tissue (GALT) system and prevent disruption of the intestinal microflora.

As shown in Fig. 1, mice with DS-induced colitis that were fed on pectin for 14 days showed a significantly higher level of stool IgA than the DS-induced colitis mice fed on cellulose. In addition, the IgA productivity during a 48-hr incubation of MLN lymphocytes was significantly higher in the mice fed on pectin than in those fed on cellulose (Fig. 2). The higher of IgA concentration in the diseased animals might reflect greater in Ig-mediated mucosa protection. In contrast, the IgE productivity in the pectin fed-groups was significantly lower than that in the cellulose-fed groups. In the case of DS-induced colitis, a similar response pattern was apparent with both pectin and cellulose feeding. Compared to the mice fed on cellulose, the IgA concentration of the mice fed on pectin was significantly higher. Since IgA plays a crucial role in preventing an allergic reaction by interfering with the allergen absorption, this effect is worth noticing. In addition, pectin enhanced the MLN IgA production more than cellulose did, and reduced the IgE production by the lymphocytes with DS-induced colitis. Thus, dietary pectin can be expected to alleviate an inflammatory reaction in the intestinal immune system.

Cytokines are important factors involved in inflammation and in regulating the immune response. IFN-γ, IL-2, TNF-α, IL-4, and IL-10 are all important in the initiation, regulation, and perpetuation of inflammation in ulcerative colitis and Crohn’s disease. We have previously reported that the effect of dietary pectin was exerted through the Th1 cell activity. In the present study, we found that, although the effect of dietary pectin on IFN-γ and IL-2 was strong, the IL-4 and IL-10 concentrations were lower in the mice with DS-induced colitis that were fed on pectin than in the cellulose-fed group. We have demonstrated that the increased cytokines from Th1 cells and the decreased Th2 cells could be reversed by treating with dietary pectin, even after persistent inflammation. These data may suggest that treating DS-induced colitis with dietary pectin would strengthen the immune system by regulating such cytokines as IL-4 and IL-10, thereby protecting against colitis-related damage. Therefore, we can state that the inhibitory effect of IL-4 and IL-10 by dietary pectin on DS-induced colitis could be mediated by the influence of IFN-γ, IL-2, and TNF-α.

Dietary fiber characteristically modifies the proportion of CD4⁺ and CD8⁺ T cells in the MLN lymphocytes. CD8⁺ T cells may regulate the development of CD4⁺ helper T-(Th) cells by producing IFN-γ or other regulatory cytokines which suppress the development of Th2 cells and favor Th1 cell growth. In the current study, dietary pectin increased the proportion of CD4⁺ and CD8⁺ T lymphocytes in MLN, which is consistent with what we have previously observed in MLN lymphocytes. As shown in Table 2, there was a significant increase of the population of CD4⁺ T cells in the DS-induced colitis group. Interestingly, the DS + pectin and...
cellulose groups were similar in normalizing the CD4+ to CD8+ ratio, indicating the effect being mediated by dietary pectin. For this reason, the effect of pectin can be expected to be mediated, at least in part, through the differentiation of T cells into Th1 cells.

Considering the response of IgA and IgE to MLN lymphocytes, it is likely that dietary pectin can alleviate the inflammation in DS-induced colitis brought about by an abnormal Th2 response. Although the exact mechanism by which dietary pectin modifies the immune indices is not apparent at present, the current observations can offer a new application of dietary pectin for an immunological role in inflammatory bowel disease.

Acknowledgment

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References

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