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Dietary Pectin Regulates the Levels of Inflammatory Cytokines and Immunoglobulins in Interleukin-10 Knockout Mice

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Pectin has protective, anti-inflammatory effects on inflammatory bowel disease (IBD), but the exact mechanism is unknown. Therefore, we investigated the immunological effect of dietary pectin in IL-10$^{-/-}$ mice, a murine model for IBD. Cytokine expression, CD4$^+$ and CD8$^+$ T cell populations, and immunoglobulin secretion were observed in three groups of mice: normal (BALb/c), IL-10$^{-/-}$, and IL-10$^{-/-}$ treated with pectin. Pectin treatment reduced expression of TNF-α and GATA-3, an important transcription factor for the Th2 immune response. These mice also expressed lower levels of IgE in the spleen and Peyer's patches (PP) and lower IgG and IgM expression in PP. Interestingly, IL-10 deficiency resulted in lower CD4$^+$ and CD8$^+$ populations in the spleen, mesenteric lymph node (MLN), and PP; however, pectin counteracted these declines in the MLN and PP. Therefore, dietary pectin downregulates the inflammatory response in the colon by moderating the production of proinflammatory cytokines and immunoglobulins.

KEYWORDS: Ulcerative colitis; inflammatory bowel disease; mesenteric lymph node; Peyer's patch; dextran sodium sulfate (DSS); IL-10

INTRODUCTION

Inflammatory bowel disease (IBD) is characterized by a dysregulated immune response of unknown etiology (*1*). IBD, including Crohn's disease (CD) and ulcerative colitis (UC), are chronic, relapsing, and remitting conditions of unknown origin with various features of immunological inflammation (*1*). IBD affects at least one in a thousand persons in many Western countries (*2*,*3*). Accumulating evidence indicates that the immune system plays a critical role in the development and perpetuation of CD and UC (*1*,*4*,*5*).

IL-10 is an important anti-inflammatory cytokine, and IL-10$^{-/-}$ mice have been used widely as a mouse model for IBD (*6*). Studies in humans have implicated impaired mucosal barrier function, pronounced innate immunity, production of proinflammatory and immune-regulatory cytokines, and the activation of CD4$^+$ T cells in the pathogenesis of IBD (*2*,*3*,*7*). Evidence indicating that cytokines initiate and perpetuate IBD in patients with CD and UC include the production of the proinflammatory cytokine tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and IL-8 at sites of inflammation (*8*−*11*).

The pectic polysaccharides, an important source of dietary fiber, are a family of structural carbohydrates that contain galacturonic acid (GalA) and are abundant in the plant cell wall (*12*). The pectic polysaccharides can be divided into the following structural classes: homogalacturonan (HG), xylogalacturonan (XGA), apiogalacturonan (AGA), rhamnogalacturonan II (RG-II), and rhamnogalacturonan I (RG-I)(*12*). There have been numerous studies documenting the benefits and anti-inflammatory properties of the dietary fibers, including pectin, in alleviating the symptoms of inflammatory bowel disease (*13*, *14*). However, the immunological role and/or mechanism by which pectin exerts its anti-inflammatory effects are poorly understood.

Previously, we reported that rats fed apple pectin had lower serum IgE levels, higher CD4$^+$/CD8$^+$ ratios, and higher interferon-γ (IFN-γ) production, particularly in the cells of the mesenteric lymph node (*15*). In a subsequent study, we found that mice fed apple pectin had lower serum IgE levels and displayed lower production of IL-4 and IL-10, presumably promoting a change in the Th1/Th2 balance toward Th1-mediated immunity in mice (*16*). Another study of colitis-associated colon cancer induced by 1,2-dimethylhydrazine (DMH) and dextran sodium sulfate (DSS) in mice demonstrated that apple pectin decreased toll-like receptor 4 (TLR4) and TNF-α expression induced by inflammation (*17*). Two other pectins, derived from cranberry (*Vaccinium oxycoccos*) and marsh cinquefoil (*Comarum palustre*), were shown to have anti-inflammatory effects on acetic acid induced colitis in mice by reducing neutrophil infiltration in sites of inflammation (*18*, *19*). Therefore, accumulating evidence supports the idea that pectin has protective, anti-inflammatory effects on IBD. However, because the exact mechanism is unknown, we investigated the immunological effect of dietary pectin on IBD in IL-10$^{-/-}$ mice.

EXPERIMENTAL PROCEDURES

Animals and Diet. Male BALB/c mice (6 weeks old, 20 to 22 g in weight) were purchased from Orient Bio Inc. (Seongnam, Gyeonggi, Korea), and male C3Bir.129P2(B6)-Il10tm1cgn/Lt IL-10$^{-/-}$ mice (6 weeks old, 17 to 20 g in weight) were purchased from Jackson Laboratory (Maine, USA). All mice were housed in specific pathogen-free conditions in the animal facility at Konkuk University. This study was approved by

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the Institutional Animal Care and Use Committee (IACUC). The mice were maintained on a 12 h dark−light cycle and allowed free access to nonpurified pellet diet (the American Institute of Nutrition AIN-76A rodent diet in nonpurified form) and tap water under controlled temperatures (25 ± 2 °C).

Apple pectin (Sigma-Aldrich, Missouri, USA) was dissolved in water and administered orally at a dose of 50 mg/kg from 1 day to 4 weeks, depending on the experimental protocol. The mice were adapted for a minimum of 7 days prior to initiation of the study. Mice were coded and randomized into 3 groups (5 in each group): normal group of BALB/c mice (no treatment), IL-10 group (IL-$10^{-/-}$ mice, no treatment), and the IL-10 + pectin group (IL-$10^{-/-}$ mice with pectin administered orally at a dose of 50 mg/kg per day up to 4 weeks). The mice were sacrificed at 11 weeks of age for further analyses.

Preparation of the Spleen, MLN and PP Lymphocyte and Cell Culture, and Measurement of the Immunoglobulins. Spleen, mesenteric lymph node (MLN) and Peyer's patch (PP) were excised from the mice, and lymphocytes were squeezed out into RPMI 1640 medium (Invitrogen, California, USA). Cell culture was a modified version of that described previously (*20*). 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 lymphocyte-mice (Cedarlane, Ontario, Canada) and centrifuged at 1500*g* for 30 min. The lymphocyte band at the interface was recovered and the cells were rinsed 3 times with RPMI 1640 medium. The lymphocytes were cultured in a 10% FBS (Invitrogen, California, USA), RPMI 1640 medium, and the immunoglobulin content of the culture supernatant was measured by ELISA (*16*). Cell viability was measured by trypan blue staining and found to be greater than 95% viable.

Measurements of IgE and IgA were by sandwich ELISA, as reported previously (*20*). To measure total immunoglobulin levels, goat anti-mouse IgE (Biosource, California, USA) and goat anti-mouse IgA (Zymed Laboratories, California, USA) were used to probe the respective immunoglobulins. These antibodies were diluted 1:1000 with 50 mM carbonate−bicarbonate buffer (pH 9.6), and each well of the 96 well plates was treated with 100 μL of the solution for 1 h at 37 °C. After blocking with 300 μL of the blocking solution for 1 h at 37 °C, wells were treated with 100 μL of the culture supernatant or mouse serum (1:100,000 dilution for IgA and 1:10 dilution for IgE) for 1 h at 37 °C. Bound IgE was then detected by reacting with biotin-conjugated mouse anti-mouse IgA and IgE followed by POD-conjugated avidin (1:5000 dilution; Dakopatts, Glastrup, Denmark) for 1 h at 4 °C. Wells were rinsed four times with 0.05% Tween 20 in PBS between each step. After incubating at 37 °C for 15 min with 100 μL of the substrate solution, the reaction was stopped by adding 100 μL of 1.5% (w/v) oxalic acid, and absorbance at 415 nm was measured with a Sunrise-Basic ELISA reader (Tecan, Männedorf, Switzerland).

Isolation of Organs Lymphocyte Subsets. To the spleen, mesenteric lymph node, and Peyer's patch lymphocytes suspended at 1×10^6 cells per 100 mL, 10% FBS/PBS was added to 5 mL of either CD4-FITC or CD8-PE monoclonal antibodies (Santa Cruz Biotechnology, California, USA), and incubated at 4 °C for 30 min. The lymphocytes were rinsed three times with PBS containing 10% FBS and centrifuged at 500*g* for 5 min. The stained lymphocytes were fixed by 2% paraformaldehyde and were counted by Epics Altra flow cytometry (Beckman Coulter, California, USA). Each analysis, including those of negative control samples, was based on at least 10,000 events after dead cells and gating on the basis of forward angle light scatter eliminated residual erythrocytes (*20*).

Western Blot Analysis. Cellular proteins were extracted from BALb/c mice and IL-10 deficient mice. 100 mg of spleen was homogenized in RIPA lysis buffer with 1% protease inhibitor cocktail using a PRO 200 homogenizer followed by sonication with a dismembrator. Homogenates were centrifuged for 15 min at 12000*g* at 4 °C. Supernatants were collected, and the cell lysates were quick frozen. The protein concentration was determined using the Bio-Rad protein assay reagent according to the manufacturer's instruction. For immunoblot analysis, 50 μg of cellular protein from treated and untreated cell extracts was transferred to a nitrocellulose (Millipore, Massachusetts, USA) membrane after resolving on a 6−12% SDS−polyacrylamide gel by electrophoresis. After transfer, the immunoblot was incubated overnight with blocking solution (5% w/v skim milk) at 4 °C, followed by incubation for 1 h with a 1:1000 dilution of polyclonal antibodies against TNF-α, IL-1β, IL-6, IL-4, interferon (IFN)-γ, STAT1, STAT6, p STAT1, p STAT6, T-bet, GATA-3 (Santa Cruz Biotechnology, California, USA, and Cell Signaling Technologies, Massachusetts, USA). The membranes were washed three times with Tween20/Tris-buffered saline (TTBS) and incubated with a 1:1000 dilution of horseradish peroxidase conjugated goat anti-rabbit IgG, donkey anti-goat and goat anti-mouse secondary antibody (Santa Cruz Biotechnology, California, USA, and Cell Signaling Technologies, Massachusetts, USA) for 1 h at room temperature. The membranes were washed again three times with TTBS and developed by enhanced chemiluminescence (GE Healthcare, Wisconsin, USA).

Table 1. Body Weight and Food Intake in Mice Fed IL-10 and/or Pectin for 4 Weeks[a]

group	initial wt (g)	final wt (g)	av wt gain (g)	food intake (g)
normal	22.20 ± 0.98 a	27.00 ± 0.63 a	4.8	17.70 ± 2.55 b
IL-10	17.60 ± 0.97 b	24.60 ± 0.63 b	7.0	19.89 ± 2.31 a
IL-10 + pectin	21.20 ± 0.45 a	23.80 ± 0.43 b	2.6	19.11 ± 2.53 a

[a] Data are mean ± SEM of 5 mice. Values denoted by different letters a−c are significantly different ($P < 0.05$).

Table 2. The Effect of IL-10 Deficiency and/or Pectin on Organ Weight and Intestinal Length[a]

	organ wt (g)		intestine length (cm)	
group	spleen	liver	small intestine	large intestine
normal	0.088 ± 0.010 b	1.412 ± 0.106 a	40.10 ± 1.95 a	10.94 ± 0.68 a
IL-10	0.154 ± 0.018 a	1.193 ± 0.100 b	37.36 ± 0.66 b	8.78 ± 0.58 b
IL-10 + pectin	0.168 ± 0.027 a	1.012 ± 0.043 c	37.74 ± 1.72 b	9.08 ± 0.46 b

[a] Data are mean ± SEM of 5 mice. Values denoted by different letters a−c are significantly different ($P < 0.05$).

Statistical Analysis. All experiments were repeated at least three times. All data were expressed as mean ± SEM values. Differences between the means of the individual groups were assessed by one-way analysis of variance with Duncan's multiple range tests. Differences were considered significant at $p < 0.05$. The statistical software package SPSS version 14.0 (SPSS Institute, Illinois, USA) was used for these analyses.

RESULTS

Body Weight, Organ Weight, and Intestinal Length. Because weight gain and total food intake can be indicators of intestinal health, we monitored body weight and food intake for each of the three groups of mice, before and after treatment (**Table 1**). The IL-10 group (IL-$10^{-/-}$ mice) displayed the most dramatic weight gain (7.0 g, **Table 1**), while the IL-10 + pectin group (IL-$10^{-/-}$ mice treated with pectin) gained the least amount of weight (2.6 g, **Table 1**). Wild-type BALB/c mice gained an average of 4.8 g during the course of the study, while also ingesting the least amount of food (17.7 g, **Table 1**). Overall, the IL-10 + pectin group gained the least amount of weight while ingesting similar or more food compared to the two other groups (**Table 1**).

We also examined the weight of the spleen and liver and measured the intestinal length in each of the three groups of mice after final treatment. Wild-type BALB/c mice had the lowest average spleen weight, highest average liver weight, and longest average small and large intestine length, all characteristics of healthy animals (**Table 2**). As expected, IL-$10^{-/-}$ mice had shorter intestinal lengths, most likely due to colitis (*6*). Also, when comparing the IL-10 and IL-10 + pectin groups, we did not observe major differences between the two groups except for the slightly lower average liver weight of the IL-10 + pectin treatment group (**Table 2**).

Effect of Pectin on Cytokine Expression in Splenic Lymphocytes. It is well-known that certain cytokines, including IFN-γ, IL-2, TNF-α, IL-4 and IL-10, are important for initiating, regulating, and perpetuating inflammation in IBD. Therefore, we assessed the expression of proinflammatory cytokines by splenic lymphocytes after pectin treatment in IL-$10^{-/-}$ mice (**Figure 1**). In the spleen,

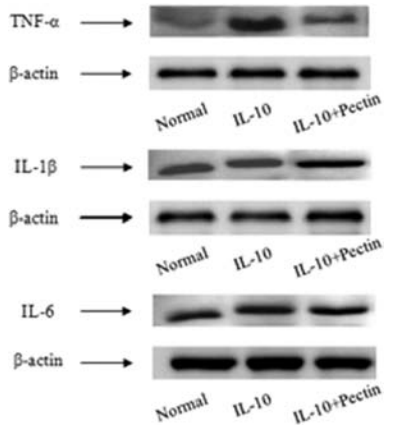

Figure 1. The effect of pectin on TNF-α, IL-1β and IL-6 expression in the mouse spleen. Expression of TNF-α, IL-1β and IL-6 protein was determined by Western blot analysis. Actin was used as an internal loading control.

expression of TNF-α was barely detectable in normal mice but much higher in the IL-10 group compared to the pectin treatment group (**Figure 1**). Significantly, IL-10 deficiency appears to trigger expression of the proinflammatory cytokine TNF-α, but its expression can be abrogated by treatment with pectin (**Figure 1**). However, IL-1β and IL-6 expression did not differ significantly among the groups (**Figure 1**).

Next, we compared Th1 and Th2 cytokine expression to determine whether there was a bias for cellular (Th1) or humoral (Th2) immune response in IBD due to IL-10 deficiency. Th1 cells promote cell-mediated immunity characterized by cellular cytolytic activity and these cells produce IFN-γ along with proinflammatory cytokines such as TNF-α(*21*). There was no significant difference in the expression level of IFN-γ in normal mice and IL-10-deficient mice (**Figure 2A**). However, T-bet, a Th1 cell-specific transcription factor that controls the expression of IFN-γ, was expressed in IL-$10^{-/-}$ mice but not normal mice (**Figure 2A**). Finally, expression of phosphorylated STAT1 (signal transducer and activator of transcription factor 1) was relatively equal among the three groups but pSTAT1 was slightly more expressed in IL-$10^{-/-}$ mice (**Figure 2A**).

The Th2 or humoral response is critical for resistance against extracellular pathogens and these cells produce IL-4, IL-5, IL-9, IL-10, and IL-13 (*21*). IL-4 and the STAT6 (signal transducer and activator of transcription factor 6) signaling pathway promote Th2 immune responses in peripheral tissues such as the skin, lung and gut (*22*). GATA-3 (member of a family of transcription factors that bind the "GATA" DNA sequence) promotes the secretion of IL-4, IL-5, and IL-13 from Th2 cells (*23*). We found that IL-4 was expressed equivalently in all three groups of mice but GATA-3 and STAT-6 expression was elevated in the IL-$10^{-/-}$ mice (**Figure 2B**). Moreover, IL-$10^{-/-}$ mice treated with pectin had reduced pSTAT6 and GATA-3 expression, suggesting that production of other Th2-related cytokines is likely to be affected (**Figure 2B**).

The Effect of Pectin on Immunoglobulin Concentration in Serum and Lymphoid Tissues. To further delineate the mechanism of action by pectin, we wanted to determine whether pectin could affect serum (IgA and IgE) and lymphoid tissue (IgA, IgE, IgG and IgM) immunoglobulin levels. IL-$10^{-/-}$ mice exhibited elevated serum IgA and IgE levels compared to normal mice, but there was no significant difference between the pectin and non-pectin groups (**Figure 3**). Next, we looked at immunoglobulin levels in the lymphoid tissues. The IgA concentration in the spleen was low for all three groups of mice and exhibited no significant

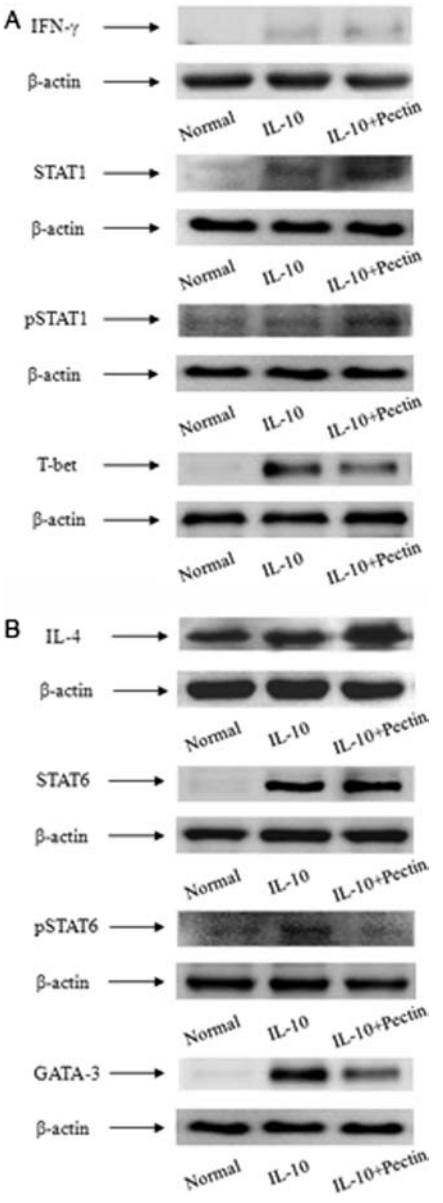

Figure 2. The effect of pectin on Th1 and Th2 cytokine expression in the mouse spleen. (**A**) Expression of IFN-γ, STAT1, pSTAT1 and T-bet protein was determined by Western blot analysis. (**B**) Expression of IL-4, STAT6, pSTAT6 and GATA-3 protein was determined by Western blot analysis. Actin was used as an internal loading control.

difference even with pectin treatment (**Figure 4A**). Interestingly, the mesenteric lymph node (MLN) and Peyer's patches (PP) of IL-$10^{-/-}$ mice had much higher IgA concentration compared to normal mice (**Figure 4A**). There was elevated IgE concentration in all lymphoid tissues of IL-$10^{-/-}$ mice and a decrease in IgE with pectin treatment in the spleen and PP (**Figure 4B**). Finally, IgG and IgM expression was highest in the spleen of IL-$10^{-/-}$ mice and declined upon pectin treatment (**Figures 4C** and **4D**). In general, IgG and IgM concentrations were higher in the MLN and PP of normal mice, compared to IL-$10^{-/-}$ mice (**Figures 4C** and **4D**). In summary, IL-$10^{-/-}$ mice displayed the most significant differences in immunoglobulin production as follows: decrease in IgE in the spleen and PP after pectin treatment, and decrease in IgG and IgM in the spleen after pectin treatment.

Effect of Pectin on the T Cell Populations within the Spleen, MLN and PP Lymphocytes. The proportion of $CD4^+$ and $CD8^+$ T cells within the lymphocyte population of the spleen, MLN, and

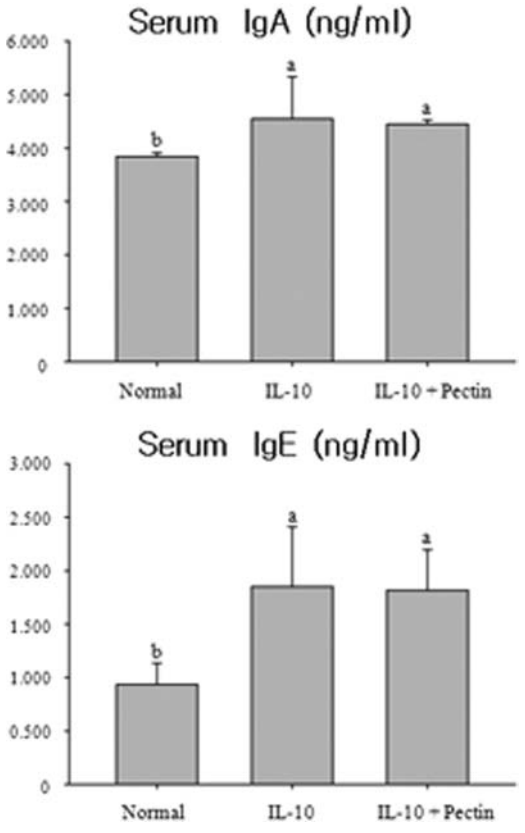

Figure 3. The effect of pectin on IgA and IgE production in serum. Antibody concentrations are in ng/mL. Data are mean ± SEM of 5 mice. Values denoted by different letters a−c are significantly different ($P < 0.05$).

PP were determined for the three groups of mice. In general, the populations of $CD4^+$ T cells and $CD8^+$ T cells in the normal group were significantly higher than those of the two other groups (**Table 3**). Also, when we measured the ratio of $CD4^+/CD8^+$ T cells in the spleen, MLN, and PP, the normal group had a higher $CD4^+/CD8^+$ T cell ratio in the spleen and lower $CD4^+/CD8^+$ T cell ratio in the MLN and PP (**Table 3**) compared to the two other groups. The higher $CD4^+/CD8^+$ T cell ratios in the MLN and PP were due to the lower percentage of $CD8^+$ cells within the MLN and PP (**Table 3**). Interestingly, there is a somewhat higher level of $CD8^+$ T cells after pectin treatment in the MLN and a significantly higher level of $CD8^+$ T cell in the PP after pectin treatment (**Table 3**). The $CD4^+$ T cells also display interesting properties in the MLN and PP. When comparing the IL-10 and IL-10 + pectin groups, pectin treatment increases the population of $CD4^+$ T cells to wild-type levels in the MLN while the $CD4^+$ T cell population is 2-fold higher in the PP (**Table 3**). Therefore, pectin treatment in IL-$10^{-/-}$ mice can increase the levels of both $CD4^+$ and $CD8^+$ cells in the MLN and PP, although there is less of an effect in the spleen. However, the $CD4^+/CD8^+$ ratio does not recover to the prototypically healthy ratio of around 2.

DISCUSSION

The observation that mice with mutations in the IL-2, IL-10, and T cell receptor (TCR) genes develop ulcerative colitis strongly suggests that IBD results from an abnormal immune response to normal antigenic stimuli (*6*, *24*, *25*). In IL-$10^{-/-}$ mice, the absence of the suppressive effect of IL-10 on the production of proinflammatory mediators by macrophages and $CD4^+$ Th1 cells results in an uncontrolled pathogenic Th1 response (*6*). That IL-10 is important for preventing intestinal inflammation is evidenced by the spontaneous colitis in *Il10rb*$^{-/-}$ and *Blimp1*$^{-/-}$ mice, both of which exhibit IL-10 production defects (*26*, *27*). Also, NF-κB expression in IL-$10^{-/-}$ mice leads to increased IL-1β, TNF-α, and IL-6 expression, thereby leading to inflammation of the intestinal mucosa (*28*). However, if NF-κB p65 (antisense oligonucleotides administered intravenously or rectally) or IKKβ expression (in macrophages and neutrophils) is downregulated, the symptoms associated with colitis improve (*28*−*30*). Thus there is a clear relationship between expression of certain cytokines and onset of inflammation within the intestinal mucosa that leads to IBD in IL-$10^{-/-}$ mice.

Here, we characterized the immunological properties of IL-$10^{-/-}$ mice suffering from colitis and we report that dietary pectin can alleviate some of the symptoms associated with intestinal inflammation. First, IL-$10^{-/-}$ mice fed dietary pectin had lower expression of TNF-α, a proinflammatory cytokine, and GATA-3, an important transcription factor for the Th2 immune response. Second, IL-$10^{-/-}$ mice fed pectin had lower IgE levels in the spleen and PP and lower IgG and IgM expression in PP. Third, IL-10 deficiency led to lower $CD4^+$ and $CD8^+$ populations in the spleen, MLN, and PP; however, pectin counteracted these declines in the intestinal lymphoid tissues.

Pectin regulates the immune response to IBD in several ways, and there are several possible repercussions for the anti-inflammatory response. For example, pectin suppresses the expression of TNF-α in IL-$10^{-/-}$ mice and this is significant because TNF-α is a major proinflammatory and anticancer cytokine (*31*). In humans, TNF-α is known to be elevated in the sera of children with active ulcerative colitis and Crohn's disease and in the stool of children with both types of IBD (*32*). Also, increased production of IL-1, TNF-α, and IL-6 is observed even in microscopically normal Crohn's disease mucosa, and cytokine profiles change during clinical evolution (*2*).

The immune response to mucosal inflammation is almost always mediated by either an excessive Th1 T cell response (excessive IL-12, IFN-γ, and TNF-α secretion) or an excessive Th2 T cell response (excessive IL-4 and IL-5 secretion), and the IL-$10^{-/-}$ mouse is a well-studied Th1 model of inflammation (*33*). However, the Th1-mediated inflammation can switch to Th2 inflammation under certain circumstances. For example, under IL-10 deficiency, Th2-mediated inflammation can result in cells where IL-4 signaling leads to GATA-3 suppression and gradual accumulation of IL-12 signaling (*26*). Therefore, it is pleasantly surprising that pectin suppresses Th2-related cytokines in IL-$10^{-/-}$ mice. The expression of the master regulator of the Th2 immune response, the GATA-3 transcription factor (*23*), was downregulated in IL-$10^{-/-}$ mice upon treatment with dietary pectin. This suggests that pectin is ideally situated as an anti-inflammatory agent as the inflammatory response switches from Th1-mediated to a Th2-mediated response in IL-$10^{-/-}$ mice. However, T-bet, a transcription factor that acts as the master regulator for Th1 immune response, was not significantly lower after pectin treatment (*21*). This observation might be significant because T-bet deficiency causes spontaneous ulcerative colitis in mice lacking an adaptive immune response and increased susceptibility to colitis in immunologically intact mice (*34*). It remains to be seen how the Th17 immune responses are affected by pectin treatment in IL-10 deficient mice.

$CD8^+$ T cells regulate the development of $CD4^+$ T cells by producing IFN-γ and other regulatory cytokines that suppress the development of Th2 cells and promote the growth of Th1 cells (*35*). Not surprisingly, IL-10-deficient mice had significantly lower populations of $CD4^+$ and $CD8^+$ T cells in the spleen, MLN, and PP. Within the intestinal lymphoid tissues of IL-$10^{-/-}$ mice, pectin had significant effect in increasing the population of $CD4^+$ and $CD8^+$ T cells; however, pectin had no effect in the spleen.

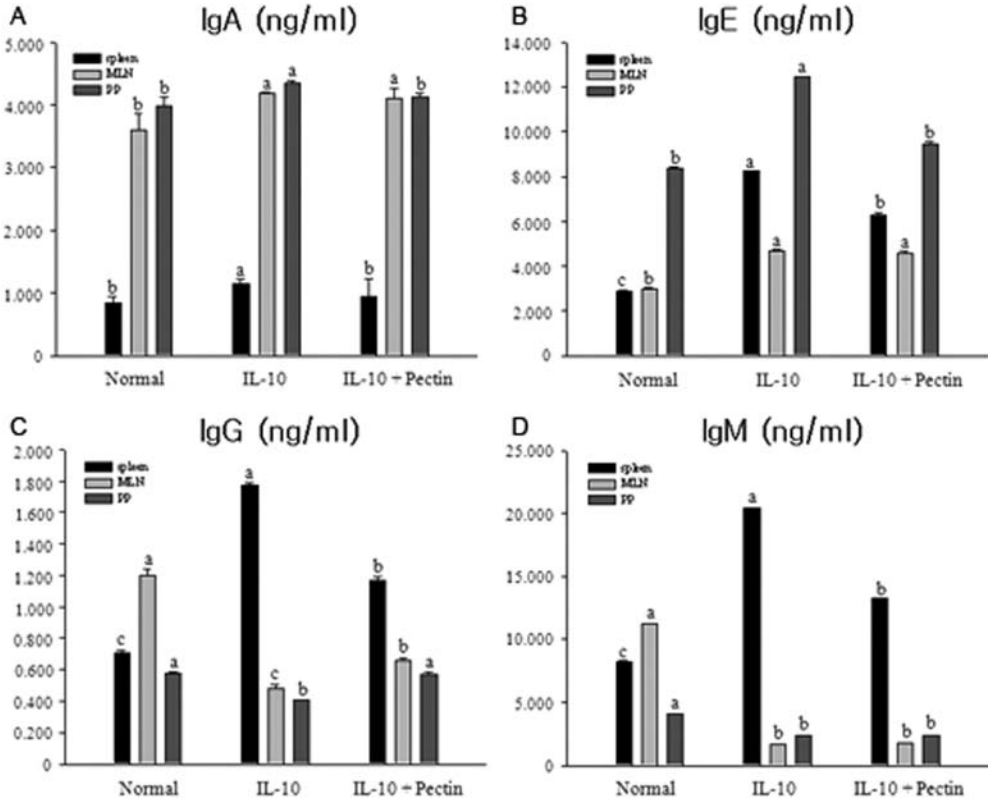

Figure 4. The effect of pectin on IgA (**A**), IgE (**B**), IgG (**C**), and Ig M (**D**) production in the mouse spleen, MLN and PP. Antibody concentrations are in ng/mL. Data are mean ± SEM of 5 mice. Values denoted by different letters a−c are significantly different ($P < 0.05$).

Table 3. The Effect of Dietary Pectin on T Cell Subsets of the Spleen, MLN and PP[a]

	spleen			MLN			PP		
group	$CD4^+$ (%)	$CD8^+$ (%)	CD4/CD8	$CD4^+$ (%)	$CD8^+$ (%)	CD4/CD8	$CD4^+$ (%)	$CD8^+$ (%)	CD4/CD8
normal	10.9 ± 1.7 a	5.2 ± 0.6 a	2.1 ± 0.1 a	28.3 ± 4.5 a	9.3 ± 1.2 a	3.0 ± 0.1 c	3.1 ± 0.8 b	2.3 ± 0.3 a	1.3 ± 0.1 c
IL-10	2.8 ± 0.8 b	1.8 ± 0.2 b	1.6 ± 0.1 b	16.7 ± 3.1 b	1.5 ± 0.7 b	11.1 ± 0.1 b	2.7 ± 0.6 b	0.4 ± 0.2 c	6.8 ± 0.1 a
IL-10 + pectin	2.4 ± 0.4 b	1.4 ± 1.2 b	1.7 ± 0.1 a	27.3 ± 1.4 a	1.8 ± 0.5 b	15.2 ± 0.1 a	6.5 ± 0.7 a	1.3 ± 0.5 b	5.0 ± 0.1 b

[a] Data are mean ± SEM of 5 mice. Values denoted by different letters a−c are significantly different ($P < 0.05$).

It is possible that pectin, by ensuring a healthier balance of $CD4^+$ and $CD8^+$ T cells, prevents overactive Th1- or Th2-mediated inflammatory response.

In humans with IBD, there is a serologic switch from an IgA-dominant to an IgG-dominant response within the intestines and the excessive production of IgG is likely to be inflammatory (*36*). There was no evidence of serological class switch in IL-$10^{-/-}$ mice as all mice had elevated IgA levels in the MLN and PP, and only slightly higher serum IgA concentration in IL-10 deficient mice. However, IL-$10^{-/-}$ mice had elevated IgG levels in the spleen that could be reduced with pectin treatment, and IL-10 deficient mice had lower IgG in the MLN compared to normal mice. Serum IgE concentration, on the other hand, was significantly higher for IL-10 deficient mice, but in the spleen and PP, pectin treatment significantly reduced IgE levels. Because IgE contributes to taming the acute inflammatory response, lower IgE concentration may prevent inflammation by activating the intestinal immune response to modulate immunoglobulin production (*37*).

The results of this study complement and further expand the knowledge gained from our earlier studies. In rats with DSS-induced colitis, the animals fed apple pectin had lower serum IgE levels, higher $CD4^+/CD8^+$ ratios, and higher IFN-γ production, particularly in the cells of the mesenteric lymph node (*15*). In a subsequent publication, we reported that mice with DSS-induced colitis had lower serum IgE levels and displayed lower production of IL-4 and IL-10 when fed dietary pectin (*16*). Also, these mice expressed lower levels of TNF-α compared to another dietary fiber, cellulose, thus affirming the anti-inflammatory ability of pectin (*16*). Here, our results demonstrate clearly that pectin moderates the immune response by downregulating proinflammatory cytokine expression, restoring $CD4^+$ and $CD8^+$ T cell populations, and lowering immunoglobulin secretion within the inflamed intestinal mucosa. However, one possible limitation of our study is that many structurally distinct pectic polymers can be produced depending upon the pectin extraction method. Ongoing efforts in our laboratory are aimed at establishing a definitive correlation between the anti-inflammatory effect of pectin and the chemical fine structure of the pectin derivative.

In conclusion, dietary pectin downregulates the inflammatory response in the intestinal mucosa by moderating the production of proinflammatory cytokines and immunoglobulins, possibly by suppressing the Th1 and/or Th2 immune responses.

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