Effects of the Oral Administration of a Probiotic Combination on the Expression of Cytokine and the Histopathology of the Large Intestine in an Animal Model of Enteritis

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Abstract

It is known that lactic-acid bacteria (LAB) helps keeping the intestine healthy and to enhance its immunologic competence. In addition, it is known to control the composition of the enterobacteria and the intestinal inflammatory reaction by inducing immunological enhancement. This study was performed, in a mouse model, to test the treatment and preventive effects of LAB of inflammatory bowel disease (IBD), which was induced by a blend of LAB-administering trinitrobenzene sulfonic acid (TNBS). To obtain the animal model of IBD, 2% TNBS was rectally administered once to a five-week-old male Balbc/J mouse. A probiotic combination was administered to the prevention group five times a week for eight weeks before the inducement of enteritis, and the mixture was administered to the treatment group five times a week, after the administration of TNBS. The changes in the levels of the cytokines of the lymph nodes and the tissue of the large intestine were observed, both with the naked eye and with a microscope. The observation showed that the levels of inflammatory cells, infiltration, and necrosis were much lower in the LAB-administered groups than in that of the control group. In addition, the inflammatory cytokines (e.g., TNF-α, IL-17A) decreased in the lymph nodes and the tissues of the large intestine. The results indicated that the administration of the combination to the animal model suppressed the inflammatory cytokines in the large intestine and in the lymph nodes, which in turn suppressed the progression of colitis.

Key words: Lactic-acid bacteria, inflammatory bowel disease, trinitrobenzene sulfonic acid, inflammatory cytokine

Introduction

There is no established treatment for inflammatory colitis, and its cause and pathophysiology are unclear. Although many studies have tried to identify the cause and pathophysiology of inflammatory colitis, the rarity of animal models with pathological conditions similar to those of inflammatory colitis limited such studies. It was reported that acetic acid or trinitrobenzene sulfonic acid (TNBS) induced colitis in rats. Particularly, the finding that TNBS induced a lesion similar to human inflammatory colitis in experimental animals accelerated studies on the pathophysiology and treatment of inflammatory colitis (Kim et al., 1996; Morris et al., 1989; Yamada et al., 1992).

Cytokine is secreted by various types of cells in the body and is involved in the proliferation, differentiation, and activation of cells. If it is in the form of polypeptides or glycoprotein, it plays a critical role in immune response and inflammatory reaction. One thing common to many etiologies of various immune disorders is the production of cytokine, which controls the immune response or damage or recovery of tissues, although the types of clinical manifestations of such immune disorders are diverse (Andreakos et al., 2002).

In patients with inflammatory intestinal disease (IBD), the levels of pro-inflammatory cytokines such as IL-1β and TNF-α are high. These cytokines are reported to play a key role in continuing and amplifying the inflammatory response of the mucous membrane. The study of the factors involved in the production and activation of such cytokines is important in the pathophysiology and treatment of IBD (Sartor, 1997). Besides, both IL-8 and macrophage colony-stimulating factor (GM-CSF) are the cytokines that can promote the release of leukocytes from the bone marrow, produce an acute phase reactant in the liver, and stimulate the vascular endothelium (Bussolino et al., 1991; Gabay and Kushner, 1999; Platzer, 1989). In particular, it is known that Th17 cells secrete IL-17A and...
IL-17F both in humans and mice, and are involved in the activation of neutrophils and in the creation of inflammatory cytokine and chemokine (Weaver et al., 2007).

It was reported that the inflammation in IBD could be triggered by the abnormal fermentation of pathogenic bacteria in the intestine, and by an imbalance between the resident bacteria. It is known that the concentration of beneficial bacteria such as lactic-acid bacteria (LAB) and bifidobacteria is low in patients with active ulcerative colitis or Crohn's disease when examined based on the histology of the colon or feces (Fabia et al., 1993; Favier et al., 1997). This suggests that maintenance of the intestinal bacterial flora or rectification of abnormal fermentation can prevent or treat IBD. Thus, this study was conducted to investigate the effects of a probiotic combination on IBD in a mouse model that was induced by TNBS, by observing the change in the expression of the inflammatory cytokine and the lesion of the colon, and by studying the possibility of the prevention and treatment of the inflammation.

Materials and Methods

Bacterial strains

The bacterial strains were Lactobacillus casei (KTCT 11863BP), Lactobacillus acidophilus (KTCT 11906BP), Streptococcus thermophilus (KTCT 11870BP), and Bifidobacterium lactis (KTCT 11903BP), and they were isolated from human feces, except for L. casei, which was isolated form a dairy product. The strains were mixed to generate a combination in the same ratio. The combination was orally administered to mice at the dose of 1×10^6 and 1×10^8 colony-forming units (CFU)/d.

Induction of colitis in the animal subjects

Five-week-old Balbc/J male mice were purchased from Central Lab. Animal Inc. Korea and were acquainted with the environment for one week. During the experiment, the temperature and humidity were kept at 23±1°C and 55±10%, respectively, with a 12 h light/dark cycle. The diet and water were provided ad libitum. This study was approved by the Animal Experiment Ethics Committee (CBTA-004) and complied with the Regulations on Animal Management. All the experiment protocols in this study were reviewed and approved by the Animal Care and Use Committee of Cellbiotech Co., Ltd. of Korea.

For the induction of colitis, a TNBS solution (2% in 50% ethanol) was introduced to the colon through the anus at the dose of 500 µL per kg of body weight, using a 5-cm long polyethylene cannula (Becton Dickinson, USA) attached to an 1 mL syringe that contained the solution. After the infusion, the mouse was positioned head-to-ground for 1 min. Ten mice were randomly assigned to four groups: the normal, control, treated, and prevented groups. The normal group was treated with 50% ethanol. The mixture was administered to the mice for eight weeks (five times a week) before the TNBS treatment (prevented group), or to the mice treated with TNBS (treated group) for one week (five times a week) after the induction of colitis by TNBS.

Collection of colon tissue samples

The mice were etherized after 12 h fasting, and then the colons were extracted. The colon was cut into three pieces, and the piece farthest from the cesium was sliced to observe the colon tissues, which were then washed three or four times with PBS at 0°C and then stored at -70°C until use. The tissue samples were fixed in a 10% formalin solution and were stored at 4°C for hematoxylin-eosin (H&E) staining.

Measurement of the cytokine mRNA levels via RT-PCR

The cytokine mRNA levels in the extracted tissues were measured via RT-PCR. The tissues were homogenized, and the RNA was separated with a TRI reagent (Sigma, USA). cDNA was synthesized with Superscript II First-strand Synthesis Kit (Invitrogen, USA), and 2 ml of the cDNA was used to determine the levels of TNF-α and IL-17A using cytokine-specific primers (Table 1).

<table>
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<tr>
<th>Genes</th>
<th>Forward sequence</th>
<th>Reverse sequence</th>
<th>Accession number</th>
<th>PCR product</th>
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<tr>
<td>TNF-α</td>
<td>CAT CTT CTC AAA ATT CGA GTG ACA A</td>
<td>TGG GAG TAG ACA AGG TAC AAC CC</td>
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<tr>
<td>IL-17A</td>
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<td>CCT TCC CTC CGC ATT GAC A</td>
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<td>GGA GAT TGT TGC CATCAA CG</td>
<td>ATG ATG ACC CTT TTG GCT CC</td>
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