Inflammatory Bowel Disease and Cytokine

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Inflammatory bowel disease, known as Crohn’s disease and ulcerative colitis, is an unexplained disease characterized by chronic inflammation that repeats a cycle of relapse, improvement, and complications. The cause of inflammatory bowel disease is not clearly known, but it is predicted that a complex of various factors precipitate its occurrence. In particular, inflammatory mediators, such as cytokine, induce an increase in cell-mediated inflammatory responses. Focal tissue damage then occurs in the intestinal mucosa because of the weakening of the immune-modulating functions of cotton. Immune and inflammatory responses do not decrease appropriately but continue until they lead to chronic inflammation. Current research has focused on the cytokine genes, which have important roles in these inflammatory responses. Cytokine is a glycoprotein that is produced mostly in activated immune cells. It connects the activation, multiplication, and differentiation between immune cells, which causes focal tissue damage and inflammatory response. Moreover, butyrate, which originates in dietary fiber and plays an important role in the structure and function of the intestinal area, shows control functions in the intestinal immune system by decreasing the proinflammatory cytokine and increasing the anti-inflammatory cytokine. Therefore, this research investigated the molecular mechanism of the anti-inflammatory effects of butyrate to comprehend the cytokine controlling abilities of butyrate in the immune cells. Butyrate is expected to have potential in new treatment strategies for inflammatory bowel disease.

**Key words**: Inflammatory bowel disease, butyrate, proinflammatory cytokine, anti-inflammatory cytokine, Th17 Cell

Inflammatory Bowel Disease

Inflammatory bowel disease, well known as the Crohn’s disease and ulcerative colitis, is a disease of unknown etiology and characterized by chronic infection which repeats of recurrence and recovery as well as the following complications [27]. In the past inflammatory bowel disease was known as a common illness in the Western hemisphere, but during the recent 10 years, the mobility rate of disease for Crohn’s disease as well as ulcerative colitis is drastically increasing all over the world [27], and the occurrence frequency is currently increasing for children and adults in domestic areas [9, 32]. Their illness leads to chronic stomachache, diarrhea, bloody excrement, and for children, important problems such as malnutrition, growth impairment, and delay in puberty take place (Fig. 1) [9, 13].

**Cause of Inflammatory Bowel Disease**

The cause of inflammatory bowel disease has not been identified clearly, but prediction has been made on how it is probably caused by complex factors which affect the epileptogenesis. These include environmental factors such as smoking or dietary, microbiological factors such as intestinal bacterial flora, immunological factors such as tissue damage due to immunological medium, and genetic factors [36, 58].

Environmental factors and genetic factors

Commonly well known environmental factors include Westernized eating habits, residence in urban areas, and cleanliness in the hygiene conditions, and a current study verified how this disease occurs frequently in those who smoke. Amongst these, a study conducted in Japan proved
how eating habit is related to disease occurrence [46]. Particularly, in terms of bacterial infection and relevance to cleanliness, it has been proven that inflammatory bowel disease is prevented when the level of cleanliness is not high [43]. The attack rate of inflammatory bowel disease is high amongst family members. When there is a patient with the Crohn’s disease or ulcerative colitis within the family, the risk of occurrence in Crohn’s disease in line family members accounts for 10–25%. Additionally, the prevalence rate is higher in biovular twins rather than uniovular twins, and as difference is shown in the prevalence rate amongst diverse racial groups, it provides support for the possibility for genetic factors [9, 14, 77].

Microbiological factor

The formational changes in the intestinal microorganisms can be identified as the representative example of microbiological factors [33]. Meanwhile, changes are happening in the formation of intestinal microorganisms and there is an increase in the number of germs in the inflammatory bowel disease [40]. Therefore, a theory is being suggested on how the incorrect immune reactions about intestinal microorganisms will set the foundation for the occurrence of inflammatory bowel disease (Fig. 2) [19, 30, 33]. The IEC barrier formed of intestinal epithelial cells (IECs) protects the lamina muscularis mucosae from intestinal microorganisms existing within the intestine. In a healthy entity, non-pathogenic commensal is enabled to live within the intestine without encountering harmful immunoreactions. In other words, it exists in the state of immune tolerance. However, in terms of an entity which holds genetic sensitivity, the intestinal functions are not able to operate properly, leading to the invasion of commensal within the internal part of the mucous membrane. The intrusive microorganism applies the PRRs (pattern recognition receptors) like TLR (toll-like receptor) and NLR (nucleotide-binding oligomerization domain (Nod)-like receptor) to response with immunocytes such as the macrophage, dendritic cell, and the neutrophil. The activation of PRR promotes the expression of proinflammatory mediators such as cytokine, chemokine, and prostaglandin and accelerates the immune reaction. As a result, it leads to the occurrence of inflammatory bowel disease [31, 83].

Immunological factor

Regardless of the cause of the occurrence, a series of processes in the inflammatory cell response due to inflammation mediator like cytokine becomes induced or amplified, or the weakening of the immunity control function leads to inducing the partial tissue damage in the intestinal mucosa [34, 67, 68]. Thus, in terms of acute inflammatory response, quick recovery generally takes due to being limited and temporary following the defense mechanism of normal hosts. However,
in the chronic inflammatory bowel disease, immunity and inflammation response do not become decreased appropriately, but continues and leads to becoming a chronic inflammation [48]. Therefore, amongst the fields of studies on inflammatory bowel disease, interest is being concentrated to factors that sustain the mediator and cellular immune response which induce the movement of neutrophil and mononuclear cell that intervene in the acute and chronic inflammatory responses.

Cytokine

Currently, interests are being concentrated to cytokine genes which are responsible for important roles in these inflammatory responses. Cytokine is the glucoprotein which mainly produce the activated immunocyte and the molecular weight is about 8-19 kD, and during immune response, it brings up partial tissue damage and inflammatory responses by relating to activation, proliferation, and division between immunocytes such as T cell, B cell, and macrophagocyte [29]. The major cells that form the cell-mediated immune system are the macrophage and T lymph cells, and macrophage produces cytokine like interleukin-1 (IL-1), IL-6, IL-8, tumor necrosis factor-alpha (TNF-α). Lymph cells are divided into CD4-T cell and CD8-T cell according to the surface antigen. CD4-T cell is distinguished into T helper (Th)1 cell and Th2 cell due to the produced cytokine, and Th1 cell mainly secretes IL-2, tumor necrosis factor-beta (TNF-β), interferon-gamma (IFN-γ) and assists in the division of cytotoxic T lymph cells. It also becomes involved in the natural form of immune response. Th2 cell secretes IL-4, IL-5, IL-6, IL-10, IL-13 and helps in the application of B lymphocyte and becomes involved in the antibody formation as well as taking the role in eliminating the external antigen [35, 50]. Moreover, the Th2 cell suppresses other cytokine through further IL-20 and takes the important role in controlling the strength of immune response. Inflammatory bowel disease sets the imbalance of immune response in Th1 and Th2 as the characteristic [4, 81], and this brings about an incongruity between the proinflammatory cytokine and anti-inflammatory cytokine and is known to play an important role in the chronicization, relapse, and practice of diseases [45, 62]. The cytokine system is being controlled through extremely complex stages, and many parts bring about cross-reaction. Since it takes part in complex interaction instead of uniform stimulation and suppression, the amount of accumulated knowledge is not as much compared to its importance [28].

Proinflammatory cytokine

Proinflammatory cytokine include the TNF-α, IL-1α and the IL-6. These control the expressions of genes involved in the cell receptor and growth components, and contains the functions which induce the cell growth and differentiation, and not only are they relevant to immune system cells, but are also secreted from various forms of tumor cell line including the keratinocyte and fibroblast, and are applied in cells through the form of autocrine
and lateral secretion [5, 18]. Additionally, they are known to increase the formation of vasculization by applying with other growth factors [39].

IL-1 is the representative cytokine which induces inflammatory response, and the expression is controlled through the NF-κB. Furthermore, IL-1 itself is also the strong factor which can activate the NF-κB. Therefore, IL-1 eventually increases the activation of NF-κB, and the expression of various proinflammatory mediators controlled by this factor and leads to inducement in excessive inflammatory response [21, 76]. As a proof supportive of this fact, the severity of inflammatory bowel disease is not only proportional to the expression degree of IL-1 [12], but the report which claims that the symptoms can be eased through the suppress of IL-1 can be stated as a form of evidence [73].

Since IL-2 is capable of maintaining the growth of T lymphocyte, which displays the cytotoxicity, for a considerable amount of period, it was referred to as the T cell growth (TCGF) existing in the culture medium of lymphocyte. From then on, IL-2 created through recombinants was applied as it was identified that activation of T cell as well as natural killer cell, NK cell, lymphocyte-activated killer (LAK) cell, B cell growth factor, and the stimulating factor of monoocyte lineage cells were evident [35].

TNF-α is a proinflammatory cytokine which is in charge of important roles in the expression and control of immune responses [36]. It gives signal to the tumor cell and causes it to kill itself, or even hides the reproduction within the virus cells. It is the signal molecule which becomes widely involved in innate immune response as it stimulates macrophage and promotes inflammatory response. This TNF-α is known to be involved in the occurrence of Alzheimer, cancer, and depression besides the inflammatory bowel disease [9]. Inflammatory bowel disease is closely related to the secretion of TNF-α, and its production was increased in the mucous membrane of patients diagnosed with the Crohn’s disease [9, 36]. When the disease first begins, TNF-α becomes produced first, and as the IL-1β increases, they stimulate each other and lead to the increase in the IL-6 [6, 69]. TNF-α includes antigen invasion in the epithelial cell of the small intestine in patients diagnosed with the Crohn’s disease and induces Th1 cytokine during the start and progress of the disease [9, 74]. Monoclonal antibodies regarding TNF-α suppresses the production of TNF-α and IFN-γ in the mononuclear cell and reduces the intestinal inflammation, and is being applied as the treatment of Crohn’s disease [75].

IFN-γ is the type II interferon, which is a type of interferon unstable in the acid, and is distinguished from the I type interferon, which is stabilized in the acid, and type I interferon includes both α and β. Production of IFN-γ becomes displayed as the T cell and NK cell becomes stimulated from the mitogen, antibody, or antigen. IFN-γ becomes involved in the antigen-presentation, macrophage activation, NK cell activation, control of IgG isotype, anti-viral activation, and inducement of nitric oxide synthase (NOS) [35].

Anti-inflammatory cytokine
Anti-inflammatory cytokine includes the IL-4, IL-5, IL-10, and the transforming growth factor-beta (TGF-β).

IL-4 was named as the B cell growth factor (BCGF) as it was identified as a molecule which stimulates the DNA synthesis of the B lymphocyte, but as it was discovered how the IL-4 molecule increased the expression of MHC class II molecules in the tissue-type B cell, it was suggested as the B cell stimulatory factor-1 (BCF-1). IL-4 is mostly produced in the T lymphocyte, mast cell, and basophil. In the B cell, IL-4 displays various vitality including the different surface molecule expression, B cell multiplication, B cell differentiation, and control of T cell activity [35].

IL-5 is secreted due to the activation of T lymphocyte which secretes the macrophage or the IL-1, IL-4, IL-6, and becomes involved in the differentiation and growth of the B lymph cell and eosinophil and applies as the important controlling element in the production, maturation, and activation of the eosinophil. Moreover, it is related to the inflammatory response of bronchial asthma, allergic disease, and autoimmune diseases. Infiltration of eosinophils related to IL-5 is frequently observed in various types of tumor tissue, and there have been research results which claim that it is related to the poor prognosis of cervical cancer [80].

IL-10 is one of the representative anti-inflammatory cytokine which is relatively produced in various cells such as Th2 cell, macrophage, activated T cell, and mast cell, and is known as the cytokine synthesis inhibitory factor which controls the inflammatory response by suppressing the secretion or gene transcription of proinflammatory cytokine such as the IL-1β, IL-12, TNF-α, IFN-γ. It takes the role of suppressing NF-κB activation within the cell and controlling the JAK-STAT signal transference system. Through this process, it suppresses the Th1 response and MHC class II antigen and promotes the life span and increase in B cells, and by promoting the production of antibodies, it displays
an anti-inflammatory application and is an essential factor in the immune regulation of the gastrointestinal area [35, 44, 55]. It is known to relatively decrease in the active inflammatory bowel disease and control the intestinal inflammation in the animal model [23, 57]. Additionally, according to recent researches, results have claimed that recovery was displayed in clinical symptoms and endoscopic improvement when IL-10 is dosed into patients with active Crohn’s disease [16, 71, 79].

**Th17 Cell**

Furthermore, during the past few years, Th17 cells producing the IL-17 and Th1/Th17 which produce the IFN-\(\gamma\) and IL-17 have been identified as the new subset of CD4-T cell [26, 65], and the pathophysiology of inflammatory bowel disease is expanding its concept from its previous Th1. Th2 cytokine unbalance into the unbalance of Th1/Th17/Treg cell [24, 59]. Th17 cell includes the IL-17, the representative cytokine, and expresses the IL-17F, IL-21 and IL-22 and gives influence to the occurrence of various immune diseases such as rheumatoid arthritis, autoimmune encephalomyelitis, and the Crohn’s disease. In particular, it is claimed to be important in the occurrence of inflammatory bowel disease, but the regulatory mechanism of inflammation is not clear [22]. However, as IL-17 becomes overly expressed in the tissue with chronic inflammation, the differentiation of Th17 cell becomes displayed as high. This is not only observed in chronic systematic inflammation, but is also found in multiple intestinal inflammatory diseases including the inflammatory bowel disease [49].

IL-17 is the cytokine which applies by setting various immune and non-immune cells as targets. IL-17 receptor is expressed in osteoblasts, fibroblasts, epithelial cells, and endothelial cells. IL-17 which combines with the IL-17 receptor causes for the stimulation in the increase of antigen-specific T cells in the inflammation area, and it increases the expression in proinflammatory substances such as the inducement of neutrophils as well as the inducement of IL-1\(\beta\) and nitric oxide synthase [41]. IL-17 induces the production of proinflammatory cytokine due to the macrophage, establishes the distance between the innate and adaptive immune system, and is particularly important for bacterial defense in the mucous membrane. Moreover, IL-17 is the inflammatory mediator of the Th17 cell differentiated from the CD4-T cell, and controls the inflammation of autoimmune diseases (Fig. 3) [1, 42]. In particular, IL-23/Th17 route is being recognized as one of the most important etiology in the inflammatory bowel disease, and various studies are being conducted about the roles of proinflammation or anti-inflammation of IL-17 [2, 3, 52]. According to recent reports, it has been identified that intestinal inflammation procedure of IL-23 processed through the Th17 route takes the role of pivotal adjustment in the Crohn’s disease (Fig. 4) [15, 27, 51].

**Treatment for inflammatory bowel disease**

Treatments for inflammatory bowel disease are mostly experiential, and are implemented with the objective of easing the inflammation or maintaining the remission. In terms of the treatment, anti-inflammatory agent called sulfasalazine or 5-aminosalicylic acid (5-ASA) are applied, or corticosteroids and immunosuppressant such as azathioprine or cyclosporine are being applied [45]. Currently, researches are being presented about the application of anti-inflammatory cytokines called the interleukin-10 (IL-10) [45, 79] or anti-tu-

![Fig. 3. T helper type 17 (Th17) formation from precursor Th0 cells and major products of Th17 cells [42].](image-url)
Fig. 4. Shifting paradigms in the pathogenesis of IBD [27].

...mor necrosis factor (TNF) called the monoclonal antibodies [11]. However, these medicines are currently frequently bringing about severe side effects. There are also problems identified with the treatment. In terms of the commonly applied corticosteroids, it is known to be very effective in suppressing almost all proinflammatory cytokine and causing improvements in the symptoms. Thus, it is very difficult or even possible to increase the amount of dosed medicine or refrain from usage. In these cases, the symptom commonly relapses during the early stage, and for the Crohn’s disease, it has been identified that patients show dependence on steroids. However, as it is well known, there is a limit to application of steroids due to numerous side effects resulting after high amounts of intake over a long period of time. Furthermore, in terms of immunosuppressant, there is a limit to application due to side effects such as bone marrow suppression or pancreatitis [38]. In cases when cytokine are directly applied, it leads to low bioavailability due to the injection of protein, and it may lead to occurrence of problems such as low pharmacokinetic and chemical instability as well as inducement of immune response [45]. Currently, as active researches are taking place about etiology of inflammatory bowel disease, cytokine and cells related to the Crohn’s disease and ulcerative colitis are being identified continually, and numerous biological medicine which selectively attacks specific molecules or routes related to the intestinal inflammation have been developed [64, 66]. Accordingly, necessity in the cytokine gene therapy is being suggested for inflammatory bowel disease, and attention is being focused in the application [45]. Therefore, researches on inflammatory mediators and cytokine amongst pathophysiology are needed in order to gain a deeper understanding about chronic inflammatory bowel disease and develop new treatment methods.

Effects of Butyrate for inflammatory bowel disease

Butyrate are mostly short-chained fatty acids which holds 4 carbons created when the carbohydrate of food within the colonic mucosa becomes digested due to bacteria [14, 70]. Butyrate is the primary source of energy of the colonic epithelial cells, and about 70% amongst the total energy consumption relies on butyrate. Additionally, the luminal concentration of butyrate can be 30 mM [10, 37, 60, 61]. As butyrate controlled the balance between the epithelial cell proliferation, differentiation, and apoptosis, it maintains the health and integrity of the intestinal area, and plays an important part for anti-cancer, anti-inflammation, and oxidative stress conditions [14, 20, 70]. Moreover, butyrate enema or high-fiber dietary increase the density of butyrate within the colon and showed outstanding effects in the inflammation treatment within the colonic mucosa [63]. For these reasons, butyrate is receiving focus as the remedy which shows possibility in treatment for the inflammatory bowel disease.

In this study, molecular mechanism about the anti-inflammatory effects of butyrate is to be comprehended through the cytokine control functions possessed by the butyrate in the immunocyte. Butyrate suppresses the LPS- and cytokine-stimulated production of proinflammatory mediators including TNF-α, IL-6. Butyrate also enhances the release of the anti-inflammatory cytokine IL-10 (Table 1) [82].

Researches on anti-inflammatory effects of butyrate which applies animal and human cells in vitro are being conducted actively. Jin-Sun Park and et., conducted the effects of butyrate in the production of proinflammatory and anti-inflammatory cytokine in the murin macrophage RAW 264.7 cells. As a result of observing the TNF-α, IL-6 and IL-10 mRNA levels by processing the sodium butyrate for 6 hours after stimulating the RAW 264.7 cells by the IFN-γ, TNF-α and IL-6 mRNA levels decreased due to the butyrate, but IL-10 mRNA levels displayed an increase. Due to these results, it can be identified that the sodium butyrate can control the cytokine expression in the transcription level (Fig. 5) [54].

Moreover, butyrate shows anti-inflammatory effects by suppressing the IL-12 and TNF-α in the human monocytes and increasing the IL-10 production. As it is displayed in
Table 1. Effect of Butyrate in the production of inflammatory mediators by isolated cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Effect observed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw 264.7 cells</td>
<td>↑ TNF-α, IL-6, NO  ↓ IL-10</td>
<td>[7, 54]</td>
</tr>
<tr>
<td>Mononuclear cells of the blood</td>
<td>↓ TNF-α</td>
<td>[78]</td>
</tr>
<tr>
<td>Monocytes and macrophages</td>
<td>↓ TNF-α</td>
<td>[17]</td>
</tr>
<tr>
<td>Monocytes</td>
<td>↓ TNF-α, IL-12, IFN-γ  ↑ IL-10</td>
<td>[63]</td>
</tr>
<tr>
<td>Rat primary microglial cells</td>
<td>↓ TNF-α, IL-6</td>
<td>[25]</td>
</tr>
<tr>
<td>Mesencephalic neuron-glia</td>
<td>↓ TNF-α</td>
<td>[9]</td>
</tr>
<tr>
<td>cultures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kupffer cells</td>
<td>↓ TNF-α</td>
<td>[56]</td>
</tr>
</tbody>
</table>

Abbreviations: interferon-γ (IFN-γ), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), tumor necrosis factor-α (TNF-α). (↑) increase and (↓) reduction.

Fig. 5. Effect of NaB on mRNA expressions of TNF-α, IL-6 and IL-10. RAW 264.7 cells were treated with IFN-γ (20 U/ml) in the absence or presence of NaB (0.5 mM) and total RNA was isolated at 6 h of IFN-γ treatment. The mRNA levels of TNF-α, IL-6, and IL-10 were determined by RT-PCR analysis. Data are representative of results obtained from two to four independent experiments [54].

Fig. 6A, the butyrate in the human monocytes stimulated by the SAC (75 μg/ml) controlled the production of proinflammatory cytokines IL-12p40 and TNF-α in a concentration-dependent manner. It became significantly controlled from the low density of 0.03 mM, and in the butyrate of 1mM, IL-12p40 became controlled to be over 90% and TNF-α controlled to about 70%. Effects of butyrate about the heterodimer IL-12p70 was also observed. As a result of observing the hormone level of heterodimer IL-12p70 after preincubation through the IFN-γ (200 U/ml) before stimulating human monocytes with the SAC (75 μg/ml), butyrate controlled this effectively, and showed identical inclination with effects regarding the IL-12p40. Furthermore, effects of butyrate regarding anti-inflammatory cytokine IL-10 were also observed. Butyrate controlled the production of IL-12 and TNF-α, but IL-10 increased significantly in the human monocytes stimulated by the SAC in a concentration-dependent manner. The density of butyrate which secreted the most amount of IL-10 was 0.25 mM (Fig. 6B). In order to find out if IL-12 secretion is related to mRNA production, the expression level of IL-12p40 and IL-12p35 mRNA was measured through the semiquantitative RT-PCR in the human monocytes stimulated by SAC and IFN-γ. Cells stimulated by the SAC and IFN-γ resulted in an increase in both IL-12p40 and IL-12p35 mRNA, and as a result of processing the byutrate together, IL-12p40 and IL-12p35 mRNA levels became controlled. As a result, it was identified that the IL-12 production was controlled as the butyrate suppressed the genetic transcription, which became involved with the IL-12 production (Fig. 7). The effects of butyrate caused on the cytokine production of human PBMC (peripheral blood mononuclear cell) was observed as well. Th1-associated cytokines IL-2 and IFN-γ became down regulated due to the butyrate (Fig. 8B), and in contrast, Th2-related cytokines IL-4 and IL-10 became up regulated in a concentration-dependent manner (Fig. 8C) [63].

Lu Liu and et., observed the effects of butyrate for cytokine secretion when LPS (1 mg/l) and butyrate (1 mmol/l) were stimulated for 24 hours in the human dendritic cell. IL-12p40 secretion caused by the LPS stimulation decreased by about 3 times due to the butyrate, and IFN-γ secretion decreased by about 5 times. In contrast, IL-10 secretion increased by about 7.5~11 times due to the butyrate (Fig. 9) [47]. In order to bring about more in-depth results based on the research results mentioned above, in vivo researches are being conducted simultaneously.
In the research conducted by Rodrigo Goulart Pacheo and et, effects caused by butyrate on the production of TNF-α, IL-1β, and TGF-β were examined through the application of colonic mucosa. Similarly as the humans' colitis, inflammation was created in rat, and butyrate was injected in the form of an enema after the enema surgery. Over the period of 8 weeks, it was inserted 2 times a week. Then, the amounts of TNF-α, IL-1β, and TGF-β were measured through ELISA in the attained colonic mucosa culture medium. The highest levels of TNF-α (Fig. 10A), IL-1β (Fig. 10B), and TGF-β (Fig. 10C) were observed in the colonic mucosa culture medium inserted with saline through a enema form, and in the group where the butyrate was processed, the amount of TNF-α, IL-1β, and TGF-β decreased significantly through a control level [53].

According to the research conducted by J-P Segain and et, inflammatory mucosal tissues produced great amounts of TNF-IL-10, IL-6 when compared to normal mucosal tissues (Fig. 11A). When the butyrate is processed here, the TNF level decreased through a butyrate concentration-dependent manner in both inflammatory (p=0.0001) and non-inflammatory/normal mucosal tissues (p=0.0153). When the 10 mM butyrate is processed, the TNF levels return to the control level. The TNF production decreases significantly if the 2 mM butyrate is processed by stimulating the TNF production after processing the LPS in the PBMC of a patient diagnosed with the Crohn's disease and a normal healthy
Fig. 8. Effect of butyrate on cytokine production in anti-CD3- stimulated PBMC. For evaluation of IL-2 and IFN-γ (A) or IL-4 and IL-10 (B), PBMC (1x10^6 cells/well) were cultured for 24 h in the presence or absence of butyrate with anti-CD3 (1 μg/ml). Supernatants were then harvested and analyzed for cytokine production by ELISA. Mean %control responses±SEM were calculated from 4 to 12 independent experiments. In unstimulated cultures cytokine levels were undetectable. Mean cytokine levels in the absence of butyrate were 1544±674 pg/ml (IL-2), 2002±488 pg/ml (IFN-γ), 123±15 pg/ml (IL-4) and 2145±583 pg/ml (IL-10). *p<0.05 for given and all higher concentrations of butyrate [63].

Fig. 9. Effects of butyrate on DCs cytokine release. Immature DCs were generated from monocytes in the absence or presence of 1 mmol/l butyrate and stimulated with LPS (1 mg/l) or butyrate (1 mmol/l) for 24 h. Then IL-12, IL-10, and IFN-γ were measured by ELISA using matched-pair antibodies. Wells from which IL-12 p40, IL-10, and IFN-γ were collected contained 1 ml culture medium with 1x10^6 DCs. Shown are the means±SD (n=5). *p<0.05 [47].

Fig. 10. Cytokine production by the colonic mucosa of the diverted segment, obtained at 8 wk. Cytokines in 24 h organ cultures were measured by enzymelinked immunosorbent assay and presented in pg/ml of culture supernatant, normalized to the protein content of tissues. (A) High levels of tumor necrosis factor-α (TNF-α) measured in saline-treated colitis were restored to normal values following treatment with glutamine or butyrate; (B), (C) The high levels of interleukin (IL)-1β and of transforming growth factor beta (TGF-β) detected in supernatants of saline-treated colitis decreased significantly after treatment with butyrate. Horizontal bars represent medians, boxes represent the 25th and 75th percentiles, and vertical bars represent ranges. Significant differences are noted (n=6 in each group) [53].
Fig. 11. Effect of butyrate on tumour necrosis factor (TNF). Colonic biopsies (A) from inflamed (n=14) or non-inflamed (n=15) mucosa of patients with Crohn's disease or from normal (n=6) mucosa of healthy controls were cultured for 24 h with or without 2 or 10 mM butyrate. **p<0.01, ***p<0.001 v 0 mM. (B) Peripheral blood mononuclear cells (PBMC) (n=7) were cultured for 24 h alone or in the presence of 2.5 μg/ml lipopolysaccharide (LPS) followed by 20 h under the same conditions with or without 2 mM butyrate. Supernatant concentrations of total TNF were assessed by WEHI cell bioassay, and results are expressed as mean (SEM). **p<0.01 v LPS [72].

Conclusion

Inflammatory bowel disease, which is characterized by chronic inflammation that repeats in relapse and improvement and other complications, has not been accurately known for its occurrence factors. However, it is perceived that various factors are in relevance, and according to previous researches, genetic and immunological conditions are being recognized as one of the important factors. Currently, researches on the correlation between cytokine genes and inflammatory bowel disease are being progressed actively, and as numerous biological medications have been developed in which they selectively attack specific molecules or routes related to intestinal inflammation, the necessity of cytokine gene therapy in treatment of inflammatory bowel disease is being suggested.

Butyrate originates from dietary fiber, which is an im-
portant substrate in the structure and functions of the intestine. As it decreases the amount of proinflammatory cytokine and increases anti-inflammatory cytokine, it shows controlling functions regarding the intestinal immune system.

From here on, it is perceived that the complicated roles of cytokine involved in the occurrence and progress of inflammatory bowel disease as well as the effects caused by butyrate on the production of cytokine will be identified gradually. If more resources on butyrate become accumulated, new treatment strategies will be suggested for the inflammatory bowel disease.

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크론병과 유양성 대장염으로 잘 알려져 있는 염증성 장질환은 계발과 호전을 반복하는 만성적인 질환인 염증 및 이에 따른 합병증을 특징으로 하는 원인 불명의 질환이다. 염증성 장질환의 발생 원인은 아직 명확히 알려져 있지 않지만 환경적인 요인과 장내 세균총과 같은 미생물학적 요인, 면역 미개에 의한 조직 손상과 같은 면역학적 요인 그리고 유전학적 요인 등이 복합적으로 발생기전에 관여 할 것이라고 추정한다. 특히 사이토카인과 같은 염증매개물질을 통해 세포매개한 염증반응의 원인의 과정이 유발 혹은 중첩되거나, 면역 조절 기능의 변화로 장 섬유의 조직 손상을 유발하게 되며 면역 및 염증 반응이 적절하지 않고 지속되어 만성 염증에 이르게 된다. 최근 이러한 염증반응에 중요한 역할을 담당하는 사이토카인 유전자에 관심이 높아지고 있다. 사이토카인은 활성화된 면역세포에서 주로 생성되는 단단백으로서 분자량이 8~10 kD 정도이며, 면역 반응 시 T세포, B세포, 대식세포 등의 면역세포 상호간에 활성화, 증식 및 분화 등에 관여하여 국소적 조직손상 및 염증반응을 일으킨다. 반면에 장의 구조와 기능에 있어 중요한 기질인 식이 섭취소에서 유래되는 Butyrate는 친염증성 사이토카인을 감소시키고 항염증성 사이토카인을 증가시킴으로써 장의 면역기능에 대한 조절기능을 보이고 있다. 따라서 본 총설에서는 Butyrate의 항염증 효과에 대한 분자적 기작을 면역세포에서 Butyrate가 가는 사이토카인 조절 능력을 통해 이해하고 Butyrate가 염증성 장질환에 대해 새로운 치료 전략을 제시 할 것으로 기대한다.