The Role of Gastrointestinal Bacterial Ecology in Inflammatory Bowel Disease (IBD)

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ABSTRACT

The pathogenesis of inflammatory bowel disease (IBD) is not yet fully understood. A genetic predisposition, some environmental factors and microbial flora of the gut are the key factors.

The presence of bacteria in the intestinal lumen is a prerequisite for the development of IBD. In animal models, mice incapable of expressing IL_2 or IL_{10} invariably develop a colitis- or Crohn-like inflammation. No inflammation occurs if they grow up in a pathogen-free environment or if they are fed with Lactobacillus sp when exposed to environmental bacteria. Thus, the absence of luminal bacteria or a different make-up there of prevents the development of inflammatory bowel disease in this model.

Patients with IBD have been found to have a decreased stool excretion of Lactobacillus and/or Bifidobacteria. Furthermore, an increased number of bacteria adherents to the mucosa and within the epithelium has been demonstrated in quantitative studies. It appears that these bacteria trigger a strong abnormal mucosal immunological response, leading to intestinal epithelial cell injury mediated by activated T-cells, mononuclear cells and macrophages. If this response can not be down regulated by regulatory T-cells, numerous inflammatory cytokines are activated by stimulation of the intracellular transcription factor NF-kB. Recently it was shown that bacterial lipopolysaccharides can activate NF-kB by binding to two specific receptors on the cell membrane (Toll-like receptors [TLR's]) or intracellular receptors (NOD's).

New insights of the role of bacteria in IBD became available by identifying susceptibility genes for IBD. Several IBD susceptibility loci were recently identified. The IBD-1 locus on chromosome 16 shows positive evidence for linkage in Crohn's disease and IBD-2 locus on chromosome 12 for ulcerative colitis. The evidence for an association with Crohn's disease at the IBD-1 locus have been shown to be attributed to mutations in the CARD15/NOD2 gene. This gene is expressed in peripheral blood monocytes and in intestinal epithelial cells and serves as a key factor of innate mucosal response to luminal bacteria as an antibacterial factor.

The intact intercellular NOD2 protein binds LPS and activates NF-kB. This activation of the NF-kB signalling pathway in response to bacterial components plays a protective role in the mucosal epithelial cells for the host against inviting pathogens and an increased apoptosis of infected cells. There is evidence, that the defective NOD2 protein variants increase the susceptibility to pathogen invasion and a decrease in cellular apoptosis.

NF-kB plays a dual role in IBD. On the mucosal epithelial cells, bacterial components bind on NOD2 proteins and protect bacterial invasion. If this barrier mechanism is not intact, the bacterial invasion stimulates via TLR- and NOD2 receptors in immune-active cells (macrophages, T-cells and monocytes) NF-kB and triggers an aberrant inflammatory response leading to tissue damage.

These new insights in the pathogenesis in IBD have led to new treatment possibilities including pre- and probiotics. These therapies are aimed at directly modulating the host immune system to suppress intestinal inflammation. This has prompted considerable interest in manipulating the enteric microenvironment as a novel therapeutic strategy. Several clinical studies showed promising results using pre- and probiotics in patients with ulcerative colitis, pouchitis and Crohn's disease. The introduction of genetically engineered probiotic organism

to produce and deliver anti-inflammatory cytokines or other biological relevant molecules to the mucosa offers further new potential for the treatment of IBD.

***Keywords** : Inflammatory Bowel Disease, inflammatory cytokines*

INTRODUCTION

Inflammatory bowel disease (IBD) includes three clinical conditions: ulcerative colitis (UC), Crohn's disease (CD) and indeterminate colitis (IC).

Specific susceptibility genes, triggering environmental (endogenous or exogenous) factors together with the intestinal flora are involved in an aberrant or up-regulated chronic immunological response, followed by inflammation of intestinal tissue.

There is expanding evidence that intestinal microorganisms may be directly involved as triggering factors.[1]. The crosstalk between intestinal bacteria and the epithelium and immunological active cells is a field of considerable scientific interest. Clinical studies have shown that treatment with pre- and probiotics have an effect on the clinical outcome in UC, CD and pouchitis.

THE ROLE OF LUMINAL BACTERIA IN IBD

Intestinal epithelial cells (IEC) are constantly exposed to bacteria and bacterial components. IEC are structural and functional barriers and serve as a frontline of host defence against microorganisms. There is growing evidence, that luminal microorganisms are involved in the development of IBD.

IL_2 gene-deficient knockout mice develop a UC-like inflammation and IL_{10} gene-deficient mice a CD-like inflammation if they are raised under normal conditions. However, if they are raised under pathogen free conditions, no inflammatory bowel disease develops.[2,3]

In the IL_{10} deficient mouse model which develop an inflammation a decreased level of Lactobacillus sp., and an increased number of bacteria adherent to or translocated into the colonic mucosa have been found. However, if these animals were raised with a supplementation of Lactobacillus plantarum, a reduction of both adherent and translocated bacteria was found, and inflammatory condition was prevented.[4]

In patients with acute UC, the number Lactobacillus sp. was reduced in the mucosa, as shown in quantitative analyses of biopsies. In patients with CD a reduction of Bifidobacteria in stool was found as compared to non-CD controls. There was no difference if the patients were in remission.[5,6] Recently, a number of studies have shown increased numbers of mucosal adherent and as well intraepithelial bacteria in patients with IBD, but not in controls.[7-10] The concentrations of mucosal bacteria increased progressively with the severity of disease, both in inflamed and non-inflamed colon. In patients with acute IBD multiple polymorphic bacteria within solitary enterocytes located next to the lamina propria have been shown. Compared to controls, the number of anaerobes (e.g. Bacteroides) were significantly higher.[7]

These data suggest that bacteria internalized by or penetrated into enterocytes are important. This may cause an aberrant mucosal immunoregulation, leading to intestinal epithelial cell injury mediated by activated T-cells, mononuclear cells and macrophages.

GENETICS IN IBD

IBD appears to be a complex genetic disorder, with multiple contributing genes. The IBD-1 linkage region on chromosome 16q contains the CD susceptibility gene CARD15/NOD2.[11] This gene is expressed in several cells (peripheral blood monocytes and mucosal epithelial cells) and serves as a key factor of innate mucosal response to luminal bacteria. The C-terminus leucine-rich repeat domain of CARD15/NOD2 serves as a pattern-recognition receptor for broad types of microbial components, such as bacterial lipopolysaccharides (LPS) and peptidoglycan (PDG).[12] The NOD2 protein serves as an intracellular receptor for LPS and PDG and this is followed by activation of the intracellular transcription factor NF-kB.

With one copy of the risk alleles, the risk to develop CD is about 2-4 fold increased; with double-dose carriage the risk increases 20-40 folds (13). If the NOD2 mutation is present, a decreased NF-kB activation in response to bacterial components was observed.[14] The innate host reaction to enteric bacteria is impaired. Thus, the greater exposure to such bacteria can trigger an aberrant T-cell inflammatory response.[15]

The IBD-2 linkage region on chromosome 12q was found more often in UC.[16]

Present research is aimed to elucidate, how such a genetically determined dysregulation of the host immune response against bacteria leads to development of clinical IBD.

IMMUNE RESPONSE IN IBD

It is suggested that the intracellular transcription factor NF-kB plays a dual role in IBD. On the mucosal epithelial cells, bacterial components bind to NOD2 proteins and protect bacterial invasion. If this barrier is disrupted, the invading bacteria stimulate via toll-like receptors (TLR's) and NOD2 receptors of macrophages, T-cells and monocytes the expression of NF-kB and trigger thereby an aberrant inflammatory response leading to tissue damage.

In CD, high levels of activated NF-kB can be found in the inflamed intestinal mucosa.[17] Bacterial products signalling TLR's may activate NF-kB. TLR4 is abundantly expressed by mononuclear cells in the epithelium and lamina propria in patients with IBD.[18] TLR4 serves as major transducing subunit of the LPS receptor complex. It is conceivable that increased LPS recognition and signalling may contribute to the enhanced NF-kB activity and subsequent synthesis of inflammatory cytokines in IBD (Fig.1).

Several genes are regulated by NF-kB and their products TNFα, IL_1, IL_6, IL_8 and IFNγ or intracellular adhesion molecule are important mediators in inflammation. Furthermore, TNFα up regulates the NOD2 protein in epithelial cells. TNFα can activate resistant macrophages, promote the release of other proinflammatory mediators and induce expression of adhesion molecules to the vascular endothelium, facilitating the migration of new inflammatory cells into the mucosa. TNFα can also contribute to the intestinal damage by directly altering the integrity of epithelial membranes. It was also shown that IECs over expressing NOD2 are sensitized to LPS and secrete high levels of chemotactic cytokine IL_8 on LPS stimulation. Thus, regulation of NOD2 expression could be an important part of the innate immune response in intestinal epithelial cells. Defective NOD2 protein variants genetically associated with Crohn's disease may further enhance inflammatory processes by perturbing epithelial barrier functions (Fig. 2).

If this impaired function persists, an uncontrolled Th1 cell activation by intracellular bacteria through the release of IL_{12} from macrophages is present with the release of pro-inflammatory cytokines (IL_2, IFNγ). On the other hand, the anti-inflammatory Th 2 cytokine response (IL_4, IL_{10}, and TGFβ) is decreased. IFNγ can facilitate the activation of resistant macrophages and the release of IL_1, IL_6 and TNFα which maintain or increase the local inflammatory response. The differentiation of Th 2 cytokine producing cells (IL_4, IL_5, and IL_{13}) is inhibited by IFNγ. It is also known that the IL_{10} down regulation of activated Th1 cells is also impaired (Fig.3).

It is important to recognize, that the mucosal immune response is different in UC and CD. There are differences in the expression of cytokines between the two diseases. In CD a Th 1 response is dominant with an increased expression of INFγ, IL_2, IL_{12} and IL_{18}.[19] This is followed by an increase of the proinflammatory cytokines TNFα, IL_1β and NF-kB.

In UC a Th 2 response with a decreased Th 1 response is more dominant. The Th 2 response increases the expression of $IL_{4, 5, 6}$ and IL_{10}.

The factor/mechanisms leading to this uncontrolled T-cell activation are not fully clarified. The loss of tolerance towards the resident bacterial flora appears to be important, and it is believed that counter-regulatory molecules (TGFβ, IL_{10}) involved in maintaining tolerance towards the resistant flora may play a role in regulating mucosal T-cell activation. It was recently shown that TGFβ1 activity is defective in IBD mucosa because of enhanced production of Smad7, an inhibitor of TGFβ1 signal transduction.[20] Furthermore, it was demonstrated, that CD mucosal T-cells display a defective apoptosis to several stimuli, associated with an increased ratio between the antiapoptotic protein Bcl-2 and the proapoptotic molecule Bax.[21]

This knowledge is the background to interfere in the microbial-host interaction with pre- and probiotics either to prevent or to treat IBD.

NEW TREATMENT OPTION FOR IBD

The role of the intestinal flora and the interaction with the intestinal immune system leading to an uncontrolled immune response in genetic susceptible patients has lead to an interest to use pre- and probiotics in patients with IBD.

There is a growing body of knowledge how pre- and probiotics interfere with the balance of pathogenic and apathogenic bacteria.

PREBIOTICS IN IBD

The most widely used prebiotics are soluble fibre which can be fermented in the colon to short chain fatty acids (SCFA); thereby stimulating the growth of beneficial bacteria (e.g. Bifidobacteria, Lactobacilli)

inhibiting the growth of harmful bacteria (e.g. Bacteroides, Clostridium difficile, E. coli).[22] SCFA's are the main energy source for colonocytes. Insufficient colonic amounts of butyrate and/or impaired oxidation by colonocytes are involved in the pathogenesis of UC.[23-26] Excessive intraluminal hydrogen sulphide by increased amounts of sulphate-reducing bacteria is proposed to impair butyrate oxidation in UC.[27] Furthermore, butyrate itself has anti-inflammatory properties by inhibiting NF-kB. It was shown in an elegant experiment that butyrate decreases TNF? and cytokine messenger RNA expression in colonic epithelial cell lines. It also diminishes the endotoxin-induced expression of cytokines by peripheral blood mononuclear cells.[28]

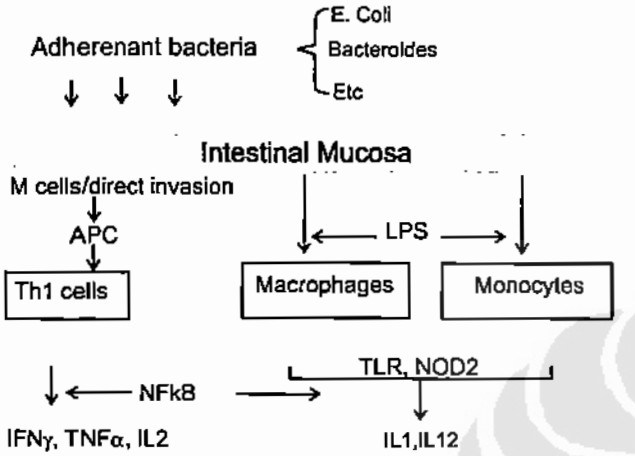

Figure 1. Bactreria and IBD: Pathogen bacteria enter through M-cells or directly in the cells. Stimulation of inflammatory cytokines by Th1-cells or via LPS by macrophages or monocytes through NF-kB.

APC= Antigen presenting cells
LPS= Lipopolysaccharides
TLR= Toll like receptors

Several clinical trials, both controlled and uncontrolled using enemas of SCFA or butyrate have recently reviewed the beneficial effect of butyrate, either given directly or produced by fermentable fibre in patients with UC.[24] Higher rates of clinical remission and a significant reduction in stool frequency have been found with topical butyrate in combination with mesalazine as compared to placebo or mesalazine alone.[29] In a small double-blind, randomized controlled trial the effect of an oral butyrate preparation to be released in the colonic lumen in combination with mesalazine compared to mesalazine alone in 25 patients with active UC was

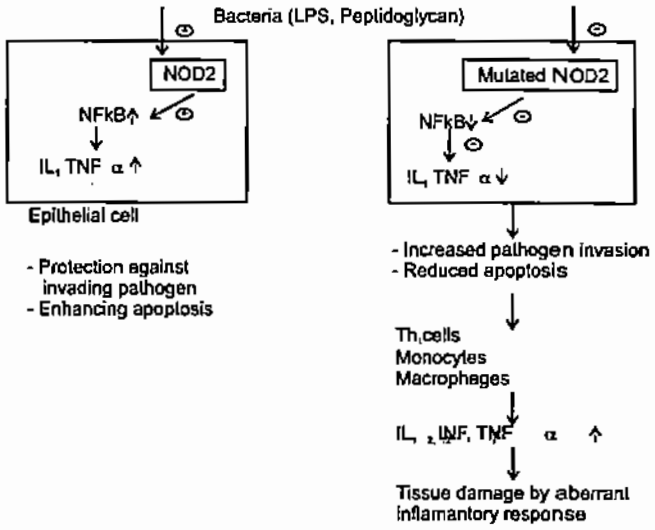

Figure 2. Mutated NOD2 impairs epithelial integrity and facilitates the invasion of bacteria in the mucosal cells, which is followed by activation of inflammatory cytokines

recently published.[30] The remission rates were similar in both groups, although a trend was observed towards a greater efficacy with butyrate,[30] Plantago ovata seeds (a slowly fermentable fibre) showed a similar one-year relapse rate compared to standard mesalazine treatment in patients with quiescent UC in another trial.[31]

PROBIOTICS AND IBD

Probiotics are living organisms, which when ingested in certain number, exert health benefits beyond inherent basic nutrition. Several probiotics (e.g. Lactobacilli, E. coli Nissle, Saccharomyces boulardii, and VSL-3 (a mixture of three strains of Bifidobacteria, four strains of Lactobacilli and one strain of Streptococcus thermophilus) were used in clinical trials.

It is known that probiotics stimulate sIgA secretion by mucosal adhesion and mucus production. Furthermore, they can stimulate phagocytosis and reduce TNF? and IFN?.

In several experimental colitis models the administration of probiotics was beneficial either in reducing inflammation or in prevention of colitis.[32-35] Madsen et al. reported that the treatment with VSL-3 brought about significant improvement of inflammation together with a reduction in mucosal levels of proinflammatory cytokines (TNF?, INF?) and a normalization of colonic physiologic function and barrier integrity in IL_{10} knockout mice.[35]

In humans, several studies in patients with UC were published using probiotics with promising results.

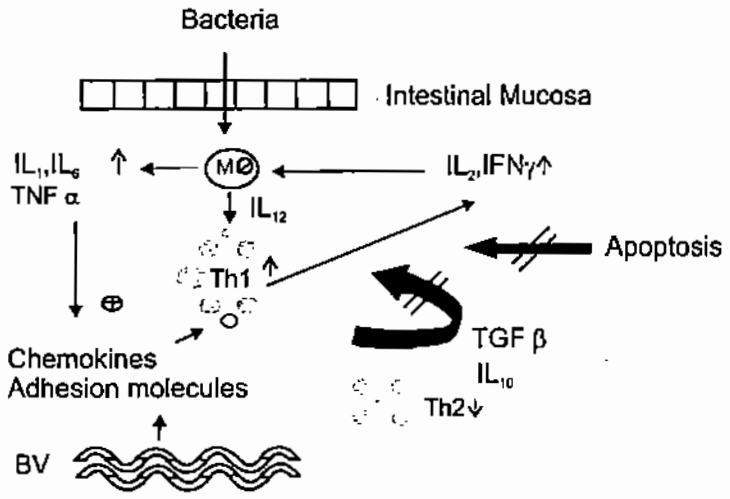

Figure 3. Bacteria and aberrant immune function: After contact of bacteria and macrophages (MØ) cytokines stimulates chemokines and adhesion molecules. This is followed by an invasion of Th cells. MØ stimulates a Th 1 response. This stimumulation is not blocked by a Th 2 action and apoptosis.

Th 1 = T effector cells
TH 2 = T regulatory cells
BV = blood vessel

A non-pathogenic strain of E. coli (Nissle) has been reported to have efficacy equivalent to that of mesalazine in preventing relapse in patients with ulcerative colitis.[36-38] On the other hand, a study with Lactobacillus GG compared to placebo was not better in maintaining postoperative remission rates in CD.[39]

The most impressive data for the efficacy of probiotics were published for the maintenance of remission or the prevention of onset in pouchitis. In the first study, 40 patients with chronic pouchitis who went into remission after 1 month of antibiotic treatment with ciprofloxacin plus rifaximin, were randomly selected to receive VSL-3 (6 g/d) or placebo for 9 months in a double-blind trial.[40] Microbiologic determination showed a notable increase in the concentration of Lactobacilli, Bifidobacteria, and Streptococcus termophilus. All 20 patients treated with placebo had a relapse during the follow-up period. In contrast, 17 of the 20 patients treated with VSL-3 were still in remission after 9 months. All these 17 patients had a relapse within 4 months after suspension of the treatment. Prolonged treatment of pouchitis with VSL-3 broad a significant increase of tissue levels of IL_{10}, a significant decrease of tissue levels of proinflammatory cytokines (TNFα, INFγ and IL_1).

In the second study Gionchetti et al[41] compared VSL-3 with placebo in a double-blind, placebo-controlled trial (1 year) in the prophylaxis of pouchitis. In the VSL-3 group (n = 20) only 2 patients had an acute pouchitis, compared to placebo with 8 of 20 patients.

Future approaches to immunotherapy in inflammatory bowel disease include genetically engineered bacteria, which secret immunosuppressive cytokines, as validated by treatment of experimental colitis by Lactococcus lactis producing IL_{10}.[42]

Although the results of these studies are exciting and intriguing, large, well-designed and well-controlled studies of pre- and probiotics are further needed.

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