ABSTRACT – Chronic inflammatory bowel diseases such as Crohn’s disease or ulcerative colitis, affect around 1 in every 1 000 individuals in western countries. They probably result from an inappropriate reaction towards the commensal microflora and are currently treated with anti-inflammatory drugs or surgery. Novel strategies aim at blocking lymphocyte recruitment and activation, improved targeting of therapeutics and modification of gut microflora. © 2001 Éditions scientifiques et médicales Elsevier SAS

1. Introduction

Human inflammatory bowel disease (IBD) is a group of intestinal inflammatory diseases that can be subdivided in ulcerative colitis (UC) and Crohn’s disease (CD) based on typical clinical manifestations. The symptoms of both are extremely unpleasant and impact all aspects of quality of life. They include diarrhea, abdominal pain, rectal bleeding, fever, nausea, weight loss, lethargy and loss of appetite. If left untreated, malnutrition, dehydration and anaemia follow, which, in extreme cases, can even lead to death. Although UC and CD show a considerable degree of similarity in etiology and epidemiology, they are entirely different in pathology. UC is restricted to the colon. CD, however, has been observed throughout the entire intestinal tract, from the mouth to the rectum. Inflammation is restricted to the mucosa in UC, whereas in CD the inflammation can be transmural, i.e. penetrating the bowel wall. This often leads to the development of perianal fistulae. An imbalance in T-helper (Th) subsets of so-called Th1 and Th2 [1] differentiates CD from UC on an immunological basis. In UC an overexpression of Th2 type cytokines (IL-4, IL-5) has been demonstrated, whereas in CD, Th1 type cytokines (IL-12, IFN-γ) predominate [2–4].

The cause of IBD is unknown. The pathogenesis of CD and UC probably involves interaction between genetic and environmental factors, such as bacterial agents (reviewed by Sartor [5]). The main theory, supported by findings in experimental models [6, 7], is that abnormal immune responses, driven by the intestinal microflora, occur in IBD. Most experimental models for IBD cannot be established in germ-free animals and IL-10−/− mice show the appearance of mucosal adherent colonic bacteria causative of the development and maintenance of the inflammation [8].

Breach of tolerance towards normal intestinal microflora may thus be the driving force behind IBD. The absence of tolerance to the indigenous microflora also appears in trinitrobenzene sulphonic acid (TNBS)-induced colitis. The administration of IL-10, a central mediator in downregulation of immune reactions, restores healthy status by re-establishing tolerance [9]. This treatment does, however, not affect immune reactivity towards heterologous bacterial antigen. Staphylococcal enterotoxin B can abrogate self-tolerance at the intestinal epithelium. IL-10 can counteract this by preventing the activation of T cells that contribute to epithelial cell damage [10]. T-cell clones stimulated by indigenous aerobic flora and bifidobacteria were also identified in patients with IBD [11].

Higher bacterial load has been reported in the mucus of IBD patients [12]. Although a number of reports measure no significant differences in the flora composition of UC patients when compared with controls, two studies indicate significant decrease of lactobacilli in UC [13, 14]. There are conflicting reports on the composition of the microflora in CD although it is difficult to compare disease stages when assessed in different centers. Bifidobacteria are found to be decreased in CD [15]. Some investigators report a significant increase in Escherichia coli and Bacteroides fragilis in the ileum and of E. coli and lacto-
bacilli in the colon [16], although lactobacilli, together with bifidobacterial scores, have also been found significantly reduced in CD patients [17]. In the latter study, abnormal fecal flora did not correlate with disease activity. In human excluded colon Eubacterium and Bifidobacterium were found reduced in favor of enterobacteria [18]. Here the altered microflora was postulated to be involved in the mucosal damage that was observed in some cases.

The development of IBD has often been proposed to be a consequence of viral or bacterial infection (Mycobacteria, Shigella, Salmonella, Yersinia, Clostridium difficile, Bacteroides vulgatus) but to date no etiological agent has been identified for IBD. Recently, however, a DNA sequence has been identified in lamina propria mononuclear cells of which the presence and serum reactivity towards the according peptide highly correlates with CD. This presently unknown sequence is not of human origin and shows homology with bacterial tetR/acrR transcription regulators [19].

It may be interesting here to bear in mind that intestinal epithelial cells (IEC), which are in close contact with the bacterial antigens present in the intestinal lumen, may act as antigen presenting cells [20, 21]. Mayer and Eisenhardt [22] demonstrated that IEC from IBD patients rather activate CD4+ T cells in contrast to healthy IEC that activate CD8+ antigen nonspecific suppressor T cells. It is therefore not surprising that evidence accumulates which attributes a key role to T cells in this mechanism of pathogenesis [23]. In normal physiology, T cells become more susceptible to programmed cell death (apoptosis) once they are activated. This eventually leads to their elimination, which is a necessary feature to allow re-establishment of the noninflamed state. In the lamina propria, T cells show increased apoptosis, even in the absence of stimulation. The theory is that this allows for stringent control of lymphocyte proliferation when immediately adjacent to such an immense pool of antigen as the intestinal content. Recent evidence shows that lamina propria T cells from CD and UC patients are more resistant to apoptosis [24–26]. Thus, IBD patients not only suffer from hyper-reactiveness towards their normal intestinal microflora but the cell populations that drive this inappropriate "auto"-immune reaction are more refractory to clearance. Alternatively, the perpetual expansion of T-cell clones that are reactive to commensal bacteria may be the very consequence of impaired apoptosis.

2. Current management of IBD

IBD represents a genuine problem in public health because of the absence of etiologic treatment. Currently a number of chemical compounds are used to revert to the noninflamed state and to maintain this remission. Corticosteroid drugs, such as the synthetic prednisone, remain the most effective treatment for active disease. These steroids have widespread actions on the immune response, and monocytes are particularly sensitive. 5-Aminosalicylic acid (5-ASA) preparations such as mesalazine and analogues are also widely used in remission therapy. Chemotherapy can be done by using azathioprine (AZA), its metabolite 6-mercaptopurine (6-MP), methotrexate and cyclosporin A. The importance of the intestinal bacterial load in the pathology of IBD allows for the use of antibiotics such as metronidazole or ciprofloxacin. Although many patients are managed successfully with conventional medical therapy, most will have recurrent activity of disease and two-thirds will require surgery.

Activated T cells can produce both anti-inflammatory and pro-inflammatory cytokines. With this knowledge in hand, IBD can be counteracted in a rational manner. Novel anti-inflammatory therapies, which make use of neutralizing monoclonal antibodies or anti-inflammatory cytokines, show the possibility of modulating the immune disregulations causative of IBD. A highly prominent and effective new therapy is systemic treatment with anti-TNF monoclonal antibodies [27]. This treatment blocks TNF, a powerful immune stimulator. Single intravenous doses, ranging from 5 to 20 mg kg⁻¹, of the cA2 infliximab antibody resulted in a drastic clinical improvement in active Crohn’s disease. Daily systemic administration of recombinant IL-10 for 7 days in doses ranging from 0.5 to 25 μg kg⁻¹ resulted in reduced Crohn’s disease activity scores and increased remission [28]. Antisense oligonucleotide therapy blocking the expression of ICAM-1 (a cell surface molecule initiating immune response upon binding) seemed to be a promising new therapy for CD [29]. The large follow-up clinical trial, however, was negative [30]. Anti-MadCAM/α4β7 integrin trials, blocking lymphocyte recruitment to the mucosa, were announced [31].

3. Novel immunological strategies

Although none of the currently available animal models covers all aspects of human IBD, they have been essential in both the understanding of the pathology and in the development of novel therapeutic strategies. A number of very promising therapies, such as the sequestering of pro-inflammatory cytokines or the prevention of T-cell infiltration, are currently emerging from such experimental models. Monoclonal antibodies to IL-12 have been shown to reduce inflammation in TNBS colitis [32] and to prevent the establishment of TNF-KLH provoked colitis in IL-2⁻/⁻ mice [33], whereas antibodies to IL-4 show a remarkable decrease in the Th2 type oxazolone-induced colitis [34]. Both monoclonal antibodies directed towards MadCAM-1 or β-7 integrin prevent recruitment of T cells to the colonic mucosa and significantly improve the pathology associated with the transfer of CD4⁺CD45RBhi T cells to SCID mice [35]. Anti-α4 integrin attenuates colitis in the cotton-top tamarin colitis model [36]. The treatment with recombinant IL-10 or anti-interferon γ monoclonal antibodies resulted, respectively, in attenuation or prevention of spontaneous colitis in IL-10⁻/⁻ mice [37]. Blocking costimulation through CD40–CD40L interaction by the addition of neutralizing mAb to CD40L resulted in clearing of TNBS colitis [38] and prevented the onset of colitis after CD4⁺CD45RBhi transfer to SCID mice [39]. Blocking the activity of the central pro-inflammatory transcription factor NF-κB by p65 antisense therapy abrogates TNBS colitis in mice [40]. Signaling following contact between
IL-6 and its soluble receptor (sIL-6R) and subsequent binding at the gp-130 is involved in T-cell resistance to apoptosis. Intersecting through the addition of neutralizing antibodies to sIL-6R or gp-130-Fc fusions cures TNBS colitis [25].

4. Novel approaches for the localized delivery of therapeutics

Long-term anti-inflammatory medication is often the fate of patients suffering from IBD. Administered orally or by injection, only a fraction of the active components of most of these drugs reaches the intended target site, the inflamed intestinal lining. This is not only an inefficient way to deliver drugs, but, more important, means that patients are often subject to a spectrum of unpleasant side effects that result from the high levels of the drugs in other, otherwise healthy tissues and organs of the body. For these reasons researchers have sought to develop methods which allow for more localized delivery of therapeutics.

The use of enemas for the localized delivery of conventional chemical therapy has gained interest in recent years [41]. This technique involves colonic instillation through a rectally introduced catheter. The pressure applied allows reaching any part of the digestive tract as required. This method, however, is very unpleasant for the patient, especially when the colon is actively inflamed, and is unsuited for delivery in a day by day routine.

Kitani et al. have designed a DNA delivery system for regulatory cytokines [42]. They engineered a plasmid that carries the transforming growth factor (TGF-β) gene under control of the CMV promoter. A single intranasal dose of the plasmid can prevent the onset or abrogate the establishment of TNBS colitis. The mechanism of action may imply the induction of regulatory (TGF-β-secreting) Th3 immune cells. Circulation of these cells through the routes of the common mucosal immune system may then allow the observed effect in distal mucosa. Most interestingly, neither prevention nor cure was observed in this model when TGF-β protein was administered through intraperitoneal injection. A drawback of this method may be the high amount of plasmid DNA required.

Exogenous IL-4 can be introduced by gene transfer using a recombinant human type 5 adenovirus (Ad5) vector [43]. Two injections of Ad5IL-4 into rats caused the overexpression of IL-4, and significantly inhibited tissue damage in TNBS-induced colitis. A single injection, however, has no effect. Gene therapy using an adenovirus–IL-10 construct was successful in preventing but not in reversing experimental colitis in the DNB rat model [44]. This method was shown only to be active when applied before or during the acute phase of induction. Moreover, when adenoviral vectors are administered through the systemic (intravenous or intraperitoneal) route this often leads to high expression of the recombinant gene in the liver and the spleen, which is highly undesirable and clearly points to a lack of organ specificity. Wirtz et al. demonstrated that rectal delivery of an adenoviral vector could lead to localized expression of the marker β-galactosidase [45]. Human trials using local liposome-mediated gene transfer of two anti-inflammatory cytokines, IL-4 and IL-10, in patients with severe inflammatory bowel disease of the rectum are planned, the rationale for the simultaneous use of IL-4 and IL-10 being to shift the immune environment in favor of Th2 immune responses [46].

We have investigated the feasibility of delivering an anti-inflammatory protein in a localized manner by the action of recombinant Lactococcus lactis. We have chosen to engineer this food-grade microorganism for the secretion of mIL-10, a major endogenous anti-inflammatory intercessor [47]. By orally administering the recombinant strain we could show 50% reduction in dextran sulfate sodium-induced colitis and prevention of colitis in IL-10−/− mice (figure 1). The method appeared as effective as steroid treatment. Comparison with treatment through intraperitoneal injection of rmIL-10 shows that a drastic reduction of the amount of therapeutic required could be achieved.

5. Probiotic therapeutics for the treatment of IBD

As discussed, conventional and experimental therapy mainly targets the inflammatory process by suppressing the host reaction. Because of the key position of the intestinal microflora in the development of IBD, manipulating this component provides a very intriguing novel therapeutic approach. As early as in 1907 Elie Metchinkoff attributed beneficial effects on human health to the consumption of Lactobacillus as present in yogurt [48]. It is a
similar concept that currently groups a number of bacterial strains known as probiotics, a term, which was introduced in 1965 by Lilly and Stillwell [49]. These bacteria are dietary components, which have a positive effect on health beyond mere nutrition, and are expected to prevent or cure (in particular intestinal) diseases. A lot of reports, however, imply vague or imprecise description of the observed phenomena. Only recently the effects of such probiotics on human and animal health were evaluated in scientifically relevant experimentation. There has indeed been a lack of well-conducted clinical and preclinical trials demonstrating any significant benefit of probiotics in humans.

Probiotic strains are often selected on an empirical basis from bacterial pools. These sources often include human isolates or traditional fermented foods. For this purpose a number of criteria have been proposed to allow more efficient and rational selection. Potentially effective probiotic bacteria are selected for properties such as bile tolerance, acid resistance, the ability of bacteria to bind to epithelial surfaces and in vitro antagonism of potentially pathogenic microorganisms. Survival and reestablishment of the bacteria in vivo appear to be linked to pH tolerance, adhesion, and antimicrobial properties in vitro [50]. An interesting intermediate in the selection of probiotic strains and appropriate formulations resides in the use of a so-called ‘simulation of the human intestinal microbial ecosystem’ (SHIME) reactor. This reactor provides access to analogs of the different compartments of the intestinal tract. An interesting and recently documented finding using SHIME is that the addition of Lactobacillus acidophilus 74-2 with fructooligosaccharide gave rise to an increase of bifidobacteria and short chain fatty acids (SCFA) [51].

Because of the lack of complexity of these in vitro systems, the beneficial characteristics of probiotics are not always transferable to the in vivo situation. An important move towards a more rational approach has emerged recently from targeted isolation of novel, probiotic strains. This aims at active management of particular diseases. This implies a second round of screening in adequate animal models for this type of disease.

An interesting development in the area of functional foods are so called prebiotics, carbohydrate oligomers that resist digestion in the upper gut. These nutrients may prove an effective means to stimulate growth of indigenous or selected probiotic strains in favor of other intestinal bacteria [52]. They can either serve as an alternative or can be complementary to and given together with probiotics.

Examples include oligofructose and inulin, which alter the human gut flora composition in favor of bifidobacteria [53].

5.1. Preclinical observations

Recent findings have shown the beneficial effects of repopulating the gut with strains of lactic acid bacteria in experimental models for IBD (table I). Madsen et al. [54] showed that in the neonatal period, IL-10−/− mice have decreased levels of Lactobacillus sp. in the colon in favor of an increase in colonic mucosal adherent and translocated bacteria. Daily intrarectal inoculation of Lactobacillus sp., or oral lactulose therapy resulted in Lactobacillus sp. repopulation, reduced colonic mucosal adherent and translocated bacteria and prevented the onset of colitis. Dunne et al. demonstrated the beneficial effects of administering probiotic combinations of Lactobacillus salivarius subsp. salivarius UCC118 and Bifidobacterium longum infantis 35624 in IL-10−/− mice [55]. Schultz et al. observed amelioration of colitis in IL-10−/− mice following continuous feeding of L. plantarum [56]. Mao et al. showed that L. plantarum and L. reuteri, preferably as a fermented oatmeal product, ameliorated methotrexate-induced colitis in rats [57]. In this study L. plantarum was more effective in reducing intestinal pathogens than L. reuteri.

5.2. Clinical evidence

Any postulated benefit on human health from consumption of probiotic bacteria can obviously only be accepted after its demonstration in clinical studies. It was not until recently that such well-designed studies have been undertaken (overview in table II). The nonpathogenic E. coli Nissle 1917 proved effective in maintenance therapy of UC: relapse rates were almost equal following administration of the live probiotic as compared to mesalazine therapy, thus offering an attractive alternative to 5-ASA treatment [58, 59]. Venturi et al. studied the effect of a combined probiotic preparation (VSL#3) of four strains of lactobacilli, three strains of bifidobacteria and Streptococcus salivarius in mesalazine intolerant UC patients [60]. Their results show that the VSL#3 preparation is effective in maintaining remission. Gianchetti et al. demonstrated the effectiveness of the VSL#3 preparation in preventing flare-ups of chronic pouchitis, an inflammatory complication that arises after ileal pouch-anal anastomosis surgery for the treatment of ulcerative colitis [61]. Administration of L. plantarum with known probiotic properties decreased pain and flatulence in patients with irritable bowel syndrome [62]. A small pilot study shows that Lactobacillus sp.
Table II. Clinical effects of probiotic strains.

<table>
<thead>
<tr>
<th>Probiotic strain</th>
<th>Clinical effect</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>E. coli</em> Nissle 1917</td>
<td>maintenance therapy of UC</td>
<td>[58–60]</td>
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<tr>
<td>VSL#3 preparation</td>
<td></td>
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<tr>
<td>VSL#3 preparation</td>
<td>decreased pain and flatulence in patients with irritable bowel syndrome</td>
<td>[61]</td>
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<tr>
<td><em>L. plantarum</em></td>
<td></td>
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<tr>
<td><em>Lactobacillus GG</em></td>
<td>increases gut barrier, improves clinical status in children with stable CD</td>
<td>[62]</td>
</tr>
<tr>
<td><em>Bifidobacterium bifidum</em></td>
<td>reduces the incidence of acute diarrhea</td>
<td>[63]</td>
</tr>
<tr>
<td><em>Streptococcus thermophilus</em></td>
<td>and Rotavirus shedding in infants</td>
<td>[64]</td>
</tr>
<tr>
<td><em>Saccharomyces boulardii</em></td>
<td>maintenance treatment of CD</td>
<td>[65]</td>
</tr>
<tr>
<td><em>Lactobacillus GG</em></td>
<td>drop in fecal TNF and α1-antitrypsin</td>
<td>[66]</td>
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GG, a probiotic strain that transiently colonizes the human intestine, possibly increases gut barrier function and improves clinical status in children with stable Crohn’s disease [63]. *Bifidobacterium bifidum* and *Streptococcus thermophilus* can reduce the incidence of acute diarrhea and *Rotavirus* shedding in infants [64]. *Saccharomyces boulardii*, a nonpathogenic yeast, proves to be interesting in the maintenance treatment of CD when combined with mesalamine [65]. In infants with atopic eczema and cow’s milk allergy *Lactobacillus GG* significantly improved dermatitis. Administration of the probiotic was associated with a drop in fecal TNF and α1-antitrypsin [66].

5.3. Possible mechanisms of probiotic activity

The possible mechanisms underlying the beneficial effects of probiotics in IBD are very diverse and interfere in most of the interactions of the host with its commensal microflora (figure 2). They may imply alteration of the microbial content and displacement of nocuous bacteria by modifying pH or producing antibacterial compounds. The latter include antibacterial peptides that are 30–60 amino acids in length and display a narrow to wide antibacterial spectrum against Gram-positive bacteria [67, 68]. Recently, new types of low-molecular-weight antimicrobial compounds were identified in the culture filtrate of *L. plantarum* VTT E-78076 [69] and nonprotein, lipophilic antibacterial activity was described in the culture supernatant of *Bifidobacterium* strains [67]. Probiotic bacteria, screened in vitro to inhibit the growth of *E. coli* O157:H7, can reduce the level of pathogen carriage in calves superinfected with *E. coli* O157:H7 [70].

Displacement of nocuous bacteria does not necessarily involve bacteriostatic or bactericidal activity but can be a consequence of physical competition for binding. Contact between invading *Salmonella typhimurium* C5 and either the culture or the supernatant of *L. casei* GG impeded the invasion by the *Salmonella* strain in Caco-2 cells, without modifying the viability of the strain. Consequently *L. casei* GG delayed the occurrence of 100% mortality in infected mice [71]. Pathogenic *E. coli* and *Salmonella* adhere to human intestinal glycoproteins extracted from feces. They can be displaced from these by a selection of probiotic strains [72]. Binding of normal fecal bacteria on immobilized mucus did not affect the subsequent adhesion of selected probiotics [73]. *L. plantarum* displays a mannose-specific adherence mechanism for binding to the human colonic cell line HT-29 [74]. Duffy describes the inhibitory effect of adherent human bifidobacterial strains against colonization by a number of diarrheagenic bacteria (*E. coli* O157; *S. typhimurium*) and viruses (murine and rhesus Rotavirus), in various in vitro and in vivo models [75].

Some bacteria have the potential to influence immune cells in a very specific way. Strain-dependant induction of cytokines after oral *Lactobacillus* administration has been described [76, 77] and may indeed provide a way to modulate local immune circuits in any desired (e.g., Th1 or Th2) direction. It is therefore interesting that the preparation VSL#3, shown to be clinically active [60, 61], has been shown to induce IL-10 production [78]. It is noteworthy that bacteria can actively interfere with the proinflammatory kxB/NFκB signaling pathway in intestinal epithelial cells by blockade of kxB-α degradation, thus adding to tolerance in the intestinal tract [79]. The composition of the fecal microflora may also have impact on the development of immunity. In infants, allergy is found associated with lower lactobacilli and higher coliforms and *Staphylococcus aureus* [80]. Feeding ovalbumin as a marker antigen, in association with selected probiotic bacteria, *L. fermentum* and *Staphylococcus carnosus*, appears to prime for an intestinal immune response [81]. This is, however, not a general aspect. Administration to humans of milk fermented by the probiotic *L. casei* strain shirota resulted in an increase of fecal lactobacilli, especially *L. casei* shirota and *Bifidobacterium* without major impact on the immune system [82] and little effect was observed on IFN-γ and IgE production associated with long-term yogurt consumption [83].

IgA secretion is known to promote the gut immunological barrier and has been shown to be of great importance in the establishment of tolerance towards the indigenous microflora in a T-cell independent manner [84]. The increase in local IgA levels resulting from ingestion of probiotics may also contribute to enhancement of the mucosal resistance against gastrointestinal infections. *Lactobacillus* GG promotes IgA-secreting plasma cell numbers in CD patients [85]. In healthy children, fecal levels of total IgA were significantly higher following intake of a formula containing viable bifidobacteria [86].

*L. plantarum* exerts beneficial effects on intestinal disorders, both in animal models and in humans [56, 57, 62]. A notable feature of *L. plantarum* is its ability to generate nitric oxide (NO) as a side product of arginine catabolism [87]. Arginine is most likely one of, if not the most impor-
NO donor in the intestine. At high concentrations, NO is a very potent proinflammatory stimulator. These high amounts are produced by inducible NO synthases as an adaptation to inflammatory stimuli. At low levels, however, as obtained from constitutive synthesis, NO proves to be a factor promoting organ integrity [88, 89]. It would therefore be possible that L. plantarum exerts probiotic effects via low-dose NO synthesis.

Aflatoxins are food and feed contaminants of fungal origin. Biidobacteria, S. aureus and E. coli were found to bind significant quantities of aflatoxin B1 [90]. Binding of aflatoxin B1 reduces the adhesion capability of L. rhamnosus strain GG and may lead to increased excretion of sequestered aflatoxin [91].

The mucus and the enterocyte lining provide a barrier that shields the intestinal immune cells from the antigen-rich luminal matrix. In a number of cases, probiotics have shown distinct effects on this barrier. The mucus is a hydrophobic, viscoelastic gel overlaying the epithelia. It is mainly composed of mucins, cross-linked by trefoil peptides. Mucins may interfere with the binding of E. coli to eucaryotic cells. Incubation of L. plantarum 299v increased MUC2 and MUC3 mRNA expression levels in HT-29 cells [92].

Disruption of the epithelial barrier leads to a large influx of antigen in the mucosa and therefore to an enhanced intestinal immune response. L. casei or Clostridium butyricum markedly enhanced gut epithelial cell proliferation (up to 200% in the colon) in rats and can thus enhance tissue repair [93]. The differences in stimulatory activity (jejunum and ileum < cecum < distal colon) most likely reflect differences in matrix flux and thus presence of the probiotic in the given areas. Long-lasting inflammation may lead to an abnormal outgrowth of the epithelium. Adenocarcinoma, a neoplastic epithelial aberration, has been found associated with UC. Biidobacterium longum or inulin administration reduces the number of colonic aberrant crypt foci induced by carcinoma azoxymethane, possibly by reducing beta-glucuronidase activity and ammonia concentration [94]. Ammonia is a putative tumor promoter produced by bacterial degradation of protein and urea. Also bacterial enzymes such as beta-glucuronidase and beta-glucosidase are thought to play a role in colon carcinogenesis.

Figure 2. Possible mechanisms of probiotic activity. Probiotic strains can displace noccuous bacteria by competitive binding (1) or killing/growth inhibition by antibacterial compounds or lowering of the pH (2). Some probiotics have defined influence on the host immune cells such as the induction of cytokine production (3) or the increase in local IgA secretion (4). Probiotic bacteria can sequester toxins and drain these from the intestine (5). Probiotics can influence epithelial and tissue integrity by low-dose NO synthesis (6), stimulation of mucus production (7), enhancing gut epithelial cell proliferation (8), inhibition of endogenous carcinogen production (9) and providing nutrients by short chain fatty acid production (10).
SCFA (mainly acetate, propionate and butyrate) are the end products of dietary carbohydrate breakdown by anaerobic bacteria in the large bowel and are the major source of energy for colonic cells [95]. SCFA deficiency has been suggested to promote colitis [96]. There are new and promising therapeutics for ulcerative colitis. The effects have been attributed to the oxidation of SCFAs in the colonocytes and to the ability of butyrate to induce effects have been attributed to the oxidation of SCFAs in the colonocytes and to the ability of butyrate to induce enzymes (i.e. transglutaminase) promoting mucosal restitution [97]. Probiotic preparations containing *Bifidobacterium, Enterococcus* or *Lactobacillus* increased total SCFA while they also slowed the net production of ammonia in pig cecal contents [98].

6. Conclusions

Conventional anti-inflammatory and immunosuppressive therapies used for the treatment of inflammatory bowel diseases are, on the whole, effective. Not all patients, however, particularly in the case of Crohn’s disease, are responsive to such treatments. The side effects of the anti-inflammatory and immunosuppressives used can also often impact quality of life to the same degree as the symptoms of the disease. Thus, the development of improved or novel strategies for the treatment of inflammatory bowel disease is a priority.

The understanding of the mucosal immune system at the molecular level and in particular in the context of IBD offers a pleiad of actors that are possible targets for novel drug development. These include soluble mediators such as cytokines, cell surface molecules such as addressins, cell adhesion molecules and co-stimulatory molecules and also the intracellular pathways directing pro-inflammatory stimuli and apoptosis. The aim of compounds that target these biologicals is to treat the imbalance of inflammatory and regulatory systems in order to restore immune homeostasis in the gut wall. The immune system, however, is a powerful, multi-potent, pleiotropic network, so that the desired intersection at one level may prove beneficial but may provoke side effects in other, up to that moment unaffected, sites. The development of tools for targeted delivery of therapeutics is therefore essential and in a way complementary to the search for such new therapeutics.

It has become clear that the microbial content of the bowel plays an important role in the onset of IBD. We are to date, however, confronted with a massive lack in our understanding of gut microbiology. Therapies that modulate the bacterial content of the intestinal tract are nevertheless being developed successfully. The desired attention from large subsidizing institutions such as the European Commission will undoubtedly provide the required foundation for profound scientific documentation.

It is fair to expect that from the combination of the above, careful selection of therapeutics and accurate delivery by a well chosen set of vector organisms will create major therapeutic opportunities for the treatment of IBD.

References


Inflammatory bowel disease


