

Current and Novel Treatments for Ulcerative Colitis

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1. Introduction

Ulcerative colitis and Crohn's disease are defined by a common term of inflammatory bowel disease. These chronic diseases result in significant morbidity and mortality. While there are no cures for these diseases, the last two decades have been a period of major advancement in our understanding of the biology of intestinal inflammation. This can be attributed to a steadily increasing number of experimental animal models with some clinical manifestation similar to those observed in human inflammatory bowel disease. These experimental animal models have also contributed greatly to our current understanding of the immunological, pathological and physiological features of chronic intestinal inflammation. However, specific causes of ulcerative colitis and Crohn's disease remain unknown. Conventional treatments for the disease include corticosteroids and immunosuppressives, however treatments in many patients are not entirely effective with many therapies associated with significant adverse effects. Thus, treatments that are effective and have little or no side effects remain an unmet need. There are numerous emerging therapeutic strategies which may be useful in the alleviation of chronic intestinal inflammation and this chapter will focus on novel therapies that may be effective for ulcerative colitis in the future.

2. Etiology of ulcerative colitis

While the precise etiology of inflammatory bowel disease is unknown, genetic susceptibility, environmental factors, impaired barrier function, imbalances or disruption to the commensal host microflora and an abnormal intestinal immune response are thought to play an important role in its manifestation.

2.1 The immune response

It is clear that not one single component of inflammatory bowel disease pathogenesis can trigger and maintain the disease. Understanding mucosal immunity in Crohn's disease and ulcerative colitis is fundamental in unraveling the complex mechanisms of chronic gut inflammation which can then provide some insight into the treatment of inflammatory bowel disease. The immune response is divided into two components, innate immunity and adaptive immunity.

2.2 Innate immunity

In the normal intestine, macrophages are conditioned by the mucosal microenvironment to express a non-inflammatory phenotype which is translated by a down-regulated expression of innate immunity receptors and constrained production of pro-inflammatory cytokines (1). In contrast, in inflammatory bowel disease-affected intestinal tissue, macrophages newly recruited from the peripheral blood still express monocyte CD14 markers but are primed for the production of various pro-inflammatory cytokines such as interleukin (IL)1 α , IL1 β , and tumour necrosis factor (TNF) α (2-3). It has been reported that in Crohn's disease these CD14⁺ pro-inflammatory macrophages are increased and subsequently result in more IL23 and TNF α production compared to controls and ulcerative colitis and contribute to the production of interferon (IFN) γ by T cells (4).

Intestinal dendritic cells (DC) are antigen-presenting cells involved in the initiation and regulation of local innate immune response but also play a role in adaptive immunity (5). Similar to macrophages, their function is dependent on the mucosal microenvironment and function to provide protection and defense, induce tolerance or mediate inflammation (6). It has been shown that in inflammatory bowel disease, intestinal DC is activated, increasing the expression of microbial receptors and production of pro-inflammatory cytokines like IL12 and IL6 (7).

2.3 Adaptive immunity

B cell immunity

There is limited focus given to B cell immunity in inflammatory bowel disease even though in active inflammatory bowel disease there is antibody production and secretion of immunoglobulin (Ig)M, IgG and IgA, by both peripheral blood and mucosal mononuclear cells (8). The patterns of antibody class production differ in ulcerative colitis and Crohn's disease; in ulcerative colitis there is a disproportional increase in IgG1 secretion, whereas in Crohn's disease IgG1, IgG2 and IgG3 are increased compared to control cells (9).

T cell immunity

There has been a considerable increase in our understanding of adaptive immunity since the identification of CD4⁺ T helper 1 (Th1) and T helper 2 (Th2) subsets in mice (10) and humans (11). The T cell immunity field is still evolving and in addition to IFN γ -producing Th1 cells and IL4, IL5 and IL13-producing Th2 cells, new Th subsets have been identified including IL17 producing Th17 cells (12) and dual IFN γ - and IL17-producing Th17 cells (13). More recently, two new subsets of CD4⁺ effector Th cells have been described, Th9 and Th22, however, their function are not clearly understood (14). Furthermore, Cosmi et al. (15) reported that Th cells can produce both IL17 and IL4 which is a dual Th17 and Th2-mediated immune response.

In addition to Th cells, another major subset is made up of T regulatory (Treg) cells whose function is to monitor the immune response and prevent an excessive and potentially harmful immune response (16-17). It has been speculated that Th17 and Treg cells share common pathways, suggesting developmental and functional links between Treg and Th17 cells (18-19). Regulatory T cells are accepted to be key players in the maintenance of tolerance and prevention of autoimmunity (20). Of particular interest are the CD4⁺ CD25^{bright} Foxp3⁺ Regulatory T cell (Treg), where mutations of the transcription factor Foxp3, crucial in the development and function of Tregs, manifest in multiple autoimmune

diseases in both mice and humans (21). In both cases a severe early onset of inflammatory bowel disease is observed as part of the pathology. A deficiency of number and/or function of Tregs are also seen in other autoimmune diseases including multiple sclerosis and systemic lupus, and the transfer of Tregs has been shown to treat experimental murine colitis and type 1 diabetes.

The Th17 effector cell is a relatively new effector cell lineage distinct from the Th1/Th2 dichotomy, and is driven by the transcription factor retinoic acid-related orphan receptor- γ t, (ROR γ t). Th17 cells secrete predominately IL17 and potentially provide critical protection against fungi and extra cellular bacteria which are not covered by Th1/Th2 immunity (22). There is good evidence that Crohn's disease has a dominant Th1 component as shown by an elevated production of IFN γ and IL12 by lamina propria mononuclear cells (23-24). As well there is an increased production of IL-17 by Th17 cells and dual IFN γ - and IL17-producing mucosal Th cells (13, 25). In contrast, ulcerative colitis is considered an atypical Th2 response based on studies demonstrating increased IL5 and IL13 production of Th cells and also IL13 by natural killer (NK) T cells in the inflamed mucosa (26). The increased production of IL13 has been shown to induce cytotoxicity and apoptosis and impair mucosal barrier function (27), which may be a contributor to the overall pathogenesis of ulcerative colitis.

3. Characteristics of ulcerative colitis

The remainder of the chapter will focus on animal models of ulcerative colitis and novel therapeutic approaches in treating this condition. The clinical symptoms of ulcerative colitis consist of severe abdominal pain and increased frequency of bloody diarrhoea. Unlike Crohn's disease, ulcerative colitis is characterized by inflammation contained to the large intestine, which affects only the mucosal layer and is superficial in comparison to the inflammation seen in Crohn's disease. Inflammation commonly extends proximally from the rectum, and extensive superficial ulceration is typical (28). Inflammation is accompanied by ulceration, edema, and hemorrhage along the length of the colon. Complications of ulcerative colitis differ from Crohn's disease, with increased risk of perforation, toxic megacolon and a higher incidence of bowel cancer. Histopathological features include the presence of neutrophil infiltrates which form crypt abscesses (29).

4. Animal models of experimental ulcerative colitis

Even with a wealth of information on the etiology of ulcerative colitis there is still no cure. As a consequence numerous animal models of ulcerative colitis have been established to elucidate the potential mechanism of ulcerative colitis and to develop therapeutic strategies within the preclinical phase.

5. Chemical-induced colitis

5.1 Dextran Sulphate Sodium(DSS)-induced colitis

Features of DSS-induced colitis

The DSS model of experimental colitis (30) is one of the most popular and widely utilized and characterized animal models of ulcerative colitis. DSS is a synthetic sulphated polysaccharide composed of dextran and sulphated anhydroglucose unit (31).

Supplementing the drinking water of rodents with low molecular weight DSS (54,000 mol. wt.) results in histopathological and symptomatic features resembling ulcerative colitis (31-32). Histologically, DSS-induced colitis resembles the damage manifested in human ulcerative colitis patients with an inflammatory response consistent with human inflammatory bowel disease (33). DSS administration also produces visual signs of disease activity including rectal bleeding, weight loss and diarrhea (32), all common features of ulcerative colitis.

The DSS-induced ulcerative colitis model has been well characterized morphologically and biochemically (34-35). After a four-day treatment with 3% DSS in the drinking water, mice show signs of acute colitis including weight loss, bloody stools, and diarrhoea (34). Histologically, DSS produces submucosal erosions, ulceration, inflammatory cell infiltration and crypt abscesses as well as epithelioglandular hyperplasia (35). The luminal bacteria in the colon induce the production of the inflammatory cytokines, IL6 and TNF α , which cause colitis. The damage induced by DSS has been reported to affect the distal colon and caecum preferentially, with lesser damage evident in the proximal colon (32). This model is particularly useful to study the contribution of the innate immune mechanism towards colitis as well as for the study of epithelial repair mechanisms.

Mechanism of action

The mouse model of colitis induced by DSS histologically resembles human ulcerative colitis, and although the exact mechanism of DSS-induced mucosal injury is not fully understood, a topical toxic effect of DSS on the colonic epithelial cells has been proposed (36). This results in loss of barrier function which would likely result in an increased uptake of luminal antigens (bacteria and bacterial products) as well as activation of lamina propria immune cells and the inflammatory response (37). It has been reported that DSS-induced colitis alters the tight junction complex resulting in the loss of barrier function thereby facilitating the development of the inflammatory infiltrate and development of intestinal inflammation (38).

Chronic model of DSS-induced colitis

DSS is commonly administered in a dose range of 3-10% for 7-10 days to induce an acute inflammation depending on the susceptibility of the species or the molecular weight of DSS (39). The DSS-induced acute colitis model may be extrapolated to a chronic colitis model by simply prolonging the administration of DSS. It has been suggested that to induce chronicity, DSS is normally administered in three to five cycles with a 1- to 2-week rest between cycles (40-41). This is useful in understanding disease progression as well as pathological inflammatory changes observed in ulcerative colitis. Interestingly, in inbred rats administered 5% DSS for 215 days, intestinal tumors (adenomas, adenocarcinomas as well as papillomas) were seen (42) predominantly in the colon and caecum.

5.2.2, 4, 6-Trinitrobenzene sulfonic acid (TNBS)-induced colitis

Features of TNBS-induced colitis

Another model that has been used widely is the well characterized haptene reagent TNBS-induced colitis. This model of chronic colitis also resembles human ulcerative colitis in its various histological features including infiltration of colonic mucosa by neutrophils and macrophages. There is also increased production of inflammatory mediators including Th1 profile of cytokines (IFN γ , TNF α and IL12) resulting in substantial inflammation and tissue

injury (43). Studies (44-45) have indicated that the TNBS-induced colitis model is useful for testing therapeutic strategies for humans. More specifically, when TNBS is introduced into the colon of susceptible mice it induces a T cell-mediated immune response within the colonic mucosa, leading to dense infiltration of T cells and macrophages throughout the entire wall of the large bowel (46). In addition, this histopathologic characteristic is accompanied by clinical features of progressive weight loss, bloody diarrhoea, rectal prolapse and large bowel wall thickening (47). The TNBS-induced colitis model has been very useful in studying many important aspects of gut inflammation, including cytokine secretion patterns, mechanisms of oral tolerance, cell adhesion and immunotherapy.

Induction of TNBS colitis

In 2001, Scheiffele and Fuss (48) described the induction of TNBS colitis in mice. Colitis is induced by the administration of TNBS through a trocar needle using a rubber catheter inserted via the anus (49). Scheiffele and Fuss (48) recommended using 0.5 to 4.0 mg TNBS in 45% to 50% ethanol intra-rectally. Inherent in this model and other similar models is the need for ethanol at high concentrations as a vehicle for intra-colonic administration of the hapten. It seems that ethanol is a prerequisite since it acts as a barrier breaker, and allows TNBS to enter the mucosa to induce colitis (50). Ethanol by itself causes severe inflammation in the intestinal mucosa therefore it is difficult to distinguish between the ethanol-induced inflammation and hapten-induced inflammation (51). There are no standard practices for this model subsequently a critical appraisal of the various studies using TNBS colitis and a recommendation for future use of this model has been extensively reviewed by te Velde et al. (52). Intra-rectal administration of TNBS results in ulceration and thickening of the bowel wall which may persist for at least 8 weeks (53). Furthermore, granulomas and Langhans-type giant cells were also observed at the site of ulceration and inflammation. The inflammation was characterized by high myeloperoxidase and decreased glutathione levels (53).

Mechanism of action

TNBS dissolved in ethanol is required to break the mucosal barrier. TNBS can bind covalently to the E-amino group of lysine and modify cell surface proteins. Colitis may develop when pre-sensitized T lymphocytes lyse hapten-modified autologous cells (54-55). T-lymphocytes will lyse hapten-modified autologous cells only if the animal has been pre-sensitized, whereas macrophages will destroy TNBS-modified autologous cells in the absence of pre-sensitization (56). In addition, TNBS may be metabolized to yield O_2^- and H_2O_2 from the interaction between ascorbate and TNBS (57) indicating that TNBS-induced colitis may partly be mediated by cytotoxic reactive oxygen species generated by the oxidative metabolism of TNBS.

A variety of inflammatory mediators may be involved in TNBS-induced colitis. The predominant arachidonate metabolites found in TNBS colitis are leukotriene B₄ (LTB₄) and the monhydroxyl fatty acids 5-HETE, 12-HETE and 15-HETE (58). The synthesis of LTB₄ increased within 4 h and peaked 24-72 h after the administration of TNBS and this increase is correlated with colonic myeloperoxidase activity (59). Furthermore, it has been shown that a significant level of luminal eicosanoids such as prostaglandin E₂ (PGE₂), 6-keto PGF₁ α , TXB₂ and LTB₄ were increased 3 days after intracolonic instillation of TNBS (59) suggesting that eicosanoids play an important role in the pathogenesis of TNBS-induced colitis. Another potential mechanism of TNBS-induced colitis may be the increased level of platelet-activating factor (PAF). High PAF production was not seen during the time of

maximal neutrophil infiltration (1-4 days after TNBS) but was seen 1-3 weeks after the induction of colitis (60). This finding suggests that PAF is unlikely to play an important role in the acute inflammatory response but may be important in the prolongation of the inflammation in this model.

Types of TNBS-induced colitis models

The TNBS-induced colitis model may be used in 3 different scenarios, (i) in acute TNBS-induced colitis in which the primary phase of the induction of Th1 response, a nonspecific inflammatory response, is analyzed (ii) established TNBS-induced colitis in which the local delayed-type hypersensitivity response is mimicked and a specific response can be analyzed and (iii) chronic TNBS-induced colitis in which repeated local induction of DTH response will lead to fibrotic lesions and a Crohn's disease-like cytokine profile (52). These forms are not well described and documented in current practices therefore it is essential to predefine the objective for using this type of experimental colitis to better understand the pathophysiology of the chosen type of colitis.

5.3 Dinitrobenzene sulfonic acid (DNBS) model of colitis

Features of DNBS-induced colitis

DNBS is another hapten which can be used to induce colonic inflammation. DNBS is less hazardous than TNBS and can be used safely in a well-ventilated room with personnel wearing protective gloves, clothing and goggles. The DNBS model produces acute and chronic inflammation and ulceration in the colon similar to TNBS (61). The feature of colitis in this model is similar to that of the TNBS model with bloody diarrhea and significant loss of body weight evident. Four days after DNBS administration, colon damage was characterized by areas of mucosal necrosis and neutrophil infiltration and the colon appeared flaccid and filled with liquid stool. The macroscopic inspection of caecum, colon and rectum showed presence of mucosal congestion, erosion, and hemorrhagic ulcerations (62). The histopathological features included a transmural necrosis and oedema and diffuse leukocyte cellular infiltrate in the submucosa.

In comparison, rats treated with DNBS have no granulomas whereas about half of the TNBS rats have granulomas (53). In the rat, DNBS causes an overproduction of nitric oxide (NO) due to induction of inducible nitric oxide synthase (iNOS), which contributes to the inflammatory process (63-64). As in the TNBS model, DNBS induces a strong inflammatory response and a significant increase in myeloperoxidase (MPO) activity compared to controls (60). Since TNBS is no longer available in the United States, DNBS can be an alternative compound for inducing experimental models of ulcerative colitis.

Induction of TNBS colitis

Colitis was induced by using a technique of acid-induced colon inflammation as described by Morris et al., (53). In fasted rats lightly anaesthetized with isoflurane, a 3.5 F catheter was inserted into the colon via the anus until the splenicflexure was reached (approximately 8 cm from the anus). 2,4-dinitrobenzenesulphonic acid (DNBS; 25 mg/rat), dissolved in 50% ethanol (total volume, 0.8 ml) was administered as an enema. While other investigator have modified the method that was first described (53), where colitis was induced in lightly anesthetized mice by an intra-rectal injection of 3 mg of DNBS in 100 µl of 50% ethanol, delivered 3 cm into the colon via a polyethylene catheter (65).

Mechanism of action

DNBS and TNBS both bind to proteins, but TNBS has an additional active nitro group and binds more readily at lower concentrations. However, DNBS is more selective and binds only to the ϵ -amino group of lysine (61).

5.4 Oxazolone-induced colitis

Features of oxazolone-induced colitis

A number of experimental models of colitis have been proposed. However, there are limited colitis models that have a Th2 profile. The oxazolone-induced colitis model is Th2-mediated and has important implications for investigating the pathogenesis and treatment of ulcerative colitis (66-67). The administration of low intrarectal doses of an inducing agent (oxazolone) in ethanol to BALB/c mice every 7 days for 10 weeks showed that in the first 3 weeks of this treatment, the mice lost about 10-15% of their starting weight and exhibited ruffled coats, hunched posture, and restricted movement. During this period 10-15% of animals died. Over the next 3 weeks the surviving mice regained weight and no longer exhibited obvious signs of chronic illness. Repetitive administration of intra-rectal ethanol alone led to a weight loss of up to 5% in the early phase of the disease (68).

Induction of oxazolone colitis

The oxazolone-induced colitis model is established by painting the skin with 0.2 ml 3% oxazolone in 100% ethanol on days 0 and 1 followed by intrarectal administration of 0.15 ml 1% oxazolone in 50% ethanol on day 7 (66). However, another study (51) used carmellose sodium/peanut oil as a non-irritating vehicle in place of ethanol and found that the oxazolone-induced colitis model was still a reproducible animal model of human colonic inflammation. The oxazolone challenge resulted in rapid development of inflammation characterized by diarrhoea, mild ulcerations, hyperemia, infiltration of inflammatory cells, epithelial damage and submucosal edema.

More recently, oxazolone-induced colitis has been established as a chronic model via repeated intrarectal administration of oxazolone in ethanol. This allows the model to be used to define specific features of the inflammatory milieu that favors tumor development (69). Chronic oxazolone-induced colitis begins as severe inflammation with corresponding weight loss, which transforms into chronic inflammation and partial weight recovery. The inflammation is marked by the rapid increase in the production of IL13 in the lamina propria and the appearance of NK T cells, which are both immunologic features of acute oxazolone-induced colitis (69). The authors also concluded that the chronic oxazolone-induced colitis model supports epithelial tumour development induced by administration of a carcinogenic agent, azoxymethane.

Mechanism of action

Similar to the TNBS-induced colitis model, oxazolone, a hapten, induces delayed-type hypersensitivity and contact hypersensitivity reactions to subsequently induce inflammation. Oxazolone-induced colitis has been suggested to be dependent on the presence of IL13 producing invariant NK T cells (70). Thus the oxazolone-induced colitis model is one of the few models suitable for the study of the Th2 dependent immune response in intestinal inflammation.

5.5 Carrageenin-induced colitis

Carrageenan is a high molecular weight sulfated polygalactan, derived from several species of red seaweeds (*Rhodophyceae*) including *Gigartina*, *Chondrus*, and *Euचेuma* (71). The most common forms of carrageenan are lambda (λ), kappa (κ), and iota (ι) (71). Carrageenans are used by the food industry to improve the texture of food products by thickening, stabilizing, or emulsifying dairy products, salad dressings, infant formulas, processed meat, soy milk, and other food products (72-73). Its use has increased markedly during the last half century, and is known to induce inflammation in rheumatological models and in intestinal models of colitis (71).

Features of carrageenan-induced colitis

Early work in animal models has demonstrated that carrageenan may cause gastrointestinal pathology, including ulcerations and tumours of the gastrointestinal tract (71-72). In guinea pigs deprived of ascorbic acid, the oral administration of degraded *E. spinosum* carrageenan induced mild to moderate colitis, while *E. cottonii* carrageenan consistently induced severe colitis. The severe colitis induced by *E. cottonii* in scorbutic animals markedly affected the mid and distal colon and showed histological changes similar to human ulcerative colitis (74). Delivery of 10% carrageen (degraded carrageenan) for 10 days in the drinking water of CF1 mice induced bloody diarrhoea and pericryptal inflammation, and produced marked dilatation of the cecum and ascending colon (75). Histologically, the mucosa was characterized by distorted crypt architecture, inflammatory infiltration of the lamina propria, and ulceration, conditions which were more pronounced in the proximal colon but were also present in the distal colon.

Induction of carrageenan colitis

Carrageenan causes a reproducible inflammatory reaction and remains a standard chemical for examining acute inflammation and effects of anti-inflammatory drugs. With or without sensitizing the animals with carrageenan, colitis is induced by supplementing the drinking water of 2-10% degraded carrageenan (74-75).

Mechanism of action

Carrageenan has been widely used to induce inflammation in experimental models of colitis in animal models (72-73), that resemble human ulcerative colitis. NF κ B is a key determinant of the intestinal epithelial inflammatory cascade and occupies a central role in the transcriptional activation of pro-inflammatory genes (76). Furthermore, Borthakur et al. (71) suggested that activation of NF κ B in the intestinal cells following carrageenan exposure is largely attributable to an increase in Bcl10. Bcl10 resides in the cytoplasm which relays receptor mediated signals to activate NF κ B (77).

6. Genetic-induced colitis

More recently, various experimental animal model of colitis, especially transgenic mice models with spontaneous colitis (78-79) have been reported and demonstrated that T cells are necessary for involvement and initiation of intestinal inflammation. New genetically engineered animals with spontaneous colitis, such as IL2 and IL10 knockout mice models, are promising tools for further understanding of the etiology of intestinal inflammation.

6.1 IL2 knockout mice

When reared and maintained under conventional specific pathogen-free conditions, IL2 deficient (IL2^{-/-}) mice spontaneously develop disorders of the hemopoietic and immune system characterized by anemia, lymphocytic hyperplasia, progressive loss of B cells, and disturbances in bone marrow hemopoietic cells. Animals that survive more than 8-9 weeks of age also develop a chronic, non-granulomatous inflammation of the colonic and caecal submucosa and mucosa. (80).

Features of IL2 deficiency-induced colitis

The histopathology of colitis observed in IL2^{-/-} mice seems to vary depending on the method of induction and the location of animal housing. The original paper (80) described the features of spontaneously developing colitis in IL2^{-/-} mice which included ulceration, crypt abscesses, destruction of the mucosal layer with epithelial dysplasia, but also mononuclear cell infiltration of the mucosa and submucosa. However, the histopathology of immunization-induced colitis and spontaneously-developing colitis in mice reared at the NIH animal facility, seem to resemble human Crohn's disease (transmural inflammation, lymphoid hyperplasia) (81). These findings suggest that the histopathological characteristics in these animals may not only be dependent on genetic but also on environmental factors.

Mechanism of action

IL2^{-/-} mice reared and maintained under gnotobiotic conditions do not develop intestinal lesions (82). Furthermore, colitis that develops in IL2^{-/-} mice under conventional conditions suggests a direct result of an abnormal immune response in the colonic mucosa to intestinal bacterial flora. It is not known specifically how the absence of IL2 accounts for colitis in IL2^{-/-} mice and the role this cytokine plays in homeostatic regulation of mucosal immunity. However, Baumgart et al. (83) suggests that IL2 is required for the generation and the function of a regulatory population of mucosal T cells or is directly involved in preventing the development of inflammatory responses to enteric antigens.

6.2 IL10 knockout mice

Only a small number of spontaneous models of chronic colitis have been employed by researchers to yield detailed information on the penetrance, severity and reproducibility of the gut inflammation (84). The most widely used of the gene-targeted models of spontaneous colitis is the IL10 deficient (IL10^{-/-}) mouse model. The IL10^{-/-} model is a well established Th1-mediated model of transmural colitis (85). IL10^{-/-} mice were generated by disrupting the IL10 gene in embryonic stem cells and although the mice were considered to have normal lymphocyte development and antibody response, growth retardation and anemia were observed (86).

Features of IL-10 deficiency-induced colitis

Mice with targeted disruption of the IL10 gene develop spontaneous pancolitis and caecal inflammation by 2-4 months of age (85). Histopathological examination of the colons obtained from mice with active disease show many of the same characteristics as those observed in human inflammatory bowel disease. The initial changes in intestinal inflammation consisted of small, focal infiltrates of inflammatory cells in the lamina propria with minimal or no epithelial hyperplasia (85, 87). Inflammatory infiltrates consisted of a mixture of lymphocytes, plasma cells, and macrophages with smaller numbers of

neutrophils and eosinophils. IL10^{-/-} mice also develop ulcers and crypt abscesses, exhibit epithelial hyperplasia, mucin production is reduced, an increased numbers of mitotic figures were observed and increased expression of major histocompatibility complex class II molecule were also observed in the intestinal epithelial cells (85-87). As mice become older, inflammation involved the submucosa or less frequently became transmural (87).

Mechanism of action

It has been demonstrated that there is a lower number of caecal bacteria observed before colitis (7 weeks of age) in IL10^{-/-} compared to C57Bl/6J mice. This suggests differences in intestinal bacteria that might be associated with the genotype which could contribute to the development of colitis in this mouse model (88).

6.3 CD45RB^{Hi} T cell transfer model of colitis

CD4⁺ T cells can be separated on the basis of their CD45RB expression into populations expressing high (CD4⁺ CD45RB^{Hi}) or low (CD4⁺ CD45RB^{Lo}) levels of this antigen (89). CD4⁺ CD45RB^{Hi} T cells isolated via fluorescence activated cell sorting from spleens of donor mice transferred to immuno-deficient SCID or RAG1/2^{-/-} recipient mice cause a wasting syndrome with transmural intestinal inflammation primarily in the colon starting 5-10 weeks after cell transfer (90-91).

Features of CD45RB^{Hi} T cell transfer model of colitis

Initial lesions consisted of minimal multifocal or diffuse inflammatory cell infiltrates in the lamina propria. In mice with more severe colitis, changes were diffuse and sometimes transmural (90). Inflammatory infiltrates consisted of macrophages and lymphocytes, accompanied by smaller numbers of neutrophils and eosinophils (90-91). Occasional multinucleated giant cells and ulcers were observed, whereas crypt abscesses were sparse (90-91). Epithelial changes included hyperplasia with lengthening and branching of glands, mucin depletion, increased numbers of mitotic figures, and enhanced levels of major histocompatibility complex class II molecule expression on intestinal epithelial cells (90).

Mechanism of action

Recipient mice repopulated with the CD⁺CD45RB^{Lo} T cell subset or both populations (CD⁺CD45RB^{Hi} and CD⁺CD45RB^{Lo} T cell subsets) do not develop colitis. CD25⁺FoxP3⁺ cells within the CD⁺CD45RB^{Lo} population account for the prevention of colitis since depletion of CD25⁺ cells from CD45RB^{Lo} cells abrogates their colitis prevention potential (92). Treg cells which produce IL-10 due to co-culture with IL-10, prevent the onset of gut inflammation and antigen-specific immune responses when transferred together with pathogenic CD4⁺CD45RB^{Hi} T cells. Furthermore, SCID mice administered both CD45RB^{Hi} T cells and Treg cells together with anti-IL10 receptor antibodies develop colitis (93). These results suggest that the progeny of CD45RB^{Hi} T cells mount a pathogenic Th1-like response in the colon of these immuno-deficient mice.

6.4 Which colitis model to use?

As increasingly more sophisticated experimental colitis models are being described and characterized, researchers have the potential to exploit the unique potential of each model to ask specific questions. No single experimental model of colitis recapitulates all of the pathogenic and clinical features of human ulcerative colitis, however each animal model has

contributed to our understanding of the mechanisms underlying initiation and perpetuation of chronic intestinal inflammation.

7. Current treatments for ulcerative colitis

At present, conventional therapies or pharmaceutical treatments have remained the mainstay of treatment for most patients suffering from ulcerative colitis. However, these treatments are variably effective with significant adverse effects and approximately 25 to 40% of patients will eventually require colectomy (94). The aim of these treatments is to induce and maintain the patient in remission. First line therapy for mild to moderate ulcerative colitis comprise of anti-inflammatory drugs containing 5-aminosalicylic acid (5-ASA), such as oral and rectal mesalamine. Sulfasalazine, the archetype for this class of medications, is cleaved upon reaching the colon releasing mesalamine. Second generation 5-ASA medications include olsalazine and balsalazide (95). Approximately 60 and 80% of patients are adequately treated with these medications and the remainder who exhibit severe ulcerative colitis are treated with a combination of corticosteroids (prednisolone). Immunosuppressives or immunomodulators such as azathioprine and mercaptopurine that treat severe inflammatory bowel disease and/or administered to patients who have inadequate response to corticosteroids can be beneficial but there is little information about their effectiveness in treating ulcerative colitis and are also associated with risks of infection and malignancy (96). Up to 20% of inflammatory bowel disease sufferers discontinue immunosuppressant therapy because of side effects (97). Biologic drugs that interfere with the inflammatory response such as the anti-TNF α agent infliximab can be effective in inducing remission for ulcerative colitis patients that are refractory to initial treatments.

It has been demonstrated that colonic bacteria may initiate inflammation of inflammatory bowel disease (97-98) and a combination therapy with antibiotics has been shown to offer significant benefit in ulcerative colitis (99-100). Most clinicians use antibiotics as an adjuvant therapy for severe ulcerative colitis despite relatively few trials conducted on their use. Although recent advances have been made in understanding the etiology and pathophysiological mechanisms underlying the pathogenesis of ulcerative colitis, the problem still remains for patients refractory to conventional treatments or not responding and being able to maintain remission effectively with maintenance treatments (101).

Currently, there is no cure for ulcerative colitis and there is increasing evidence that alternative therapies (102-103) may provide some insights into developing a potential successful treatment. The remainder of this chapter will focus on the potential therapeutic interventions which target various aspects of the etiology of ulcerative colitis including the use of pre- and probiotics to manipulate the gut microflora and molecules that mediate the action of inflammatory cells (104). Biological therapies for ulcerative colitis will not be covered in this chapter as this area is reviewed by Rutgeerts et al. (105).

8. Novel therapies for ulcerative colitis

8.1 n-3 fatty acids

Over the years, dietary n-3 fatty acids have gained a reputation in preventing and treating several disorders including cardiovascular diseases, rheumatoid arthritis and Alzheimer's disease by way of anti-inflammatory, antithrombotic, antiarrhythmic, hypolipidemic and vasodilatory activities (101, 106-111). It has been shown in human and animal studies that

these n-3 fatty acids have potent immunomodulatory and anti-inflammatory effects by inhibiting the production of inflammatory mediators, eicosanoids, PGE2 and LTB4 and cytokines, TNF α and IL1 β (112). It stands to reason that supplemental n-3 fatty acids might therefore be beneficial in treating or preventing relapse in chronic inflammatory diseases such as ulcerative colitis (113).

Animal studies

There have been numerous studies utilising experimentally induced colitis animal models to define the role of dietary n-3 fatty acids in disease prevention and progression. In a severe combined immunodeficient (SCID) mouse model of colitis, Whiting et al. (114) found that dietary n-3 fatty acids reduced clinical colitis and colonic immunopathology by decreasing the synthesis of proinflammatory cytokines, reducing myeloid cell recruitment and activation, and enhancing epithelial barrier function and mucosal wound healing mechanisms. Li et al. (115) demonstrated within the TNBS rat colitis model that rats pretreated with n-3 fatty acids showed significant attenuation of colonic injury and protection. Compromised epithelial barrier in ulcerative colitis by chronic immune cell activation might be explained by the altered expression and distribution of tight junction proteins in tight junction membrane microdomains of the intestinal mucosa, and n-3 fatty acids have been shown to positively affect this altered expression and distribution (115). Many studies have shown adiponectin, a protein hormone produced and secreted primarily by adipocytes and more recently by colonic myofibroblasts to play a beneficial role in ulcerative colitis due to its anti-inflammatory effect (116-118). Interestingly, Matsunaga et al. (119) who found a decrease in adiponectin expression in DSS-induced colitic mice also found a further decrease in adiponectin expression in colitic mice fed with n-3 fatty acids, which could have contributed to the observed exacerbated colitis for this group. This is in contrast to the beneficial effects other studies have shown regarding dietary n-3 fatty acids and colitis.

Human studies

Fish oil is the best source of n-3 fatty acids. Although numerous studies have focused on oral supplementation in patients with inflammatory bowel disease which ultimately results in the incorporation of n-3 fatty acids into the gut mucosal tissue thereby modifying inflammatory mediators (120-121), the evidence of clinical benefits remains unclear due to conflicting results. A systematic review of the effects of fish oil in human ulcerative colitis by MacLean et al. (113) found significant improvements in clinical scores in three studies at one or more time points relative to the comparative study arm. Studies that were restricted to patients with ulcerative colitis (122-123) reported a statistical improvement in the endoscopic score with fish oil relative to comparative treatment. Together with studies that observed induction of remission (122, 124), prevention of relapse (124-128) and the requirement for immunosuppressive agents (122, 124, 129). MacLean et al. (113) deduced that there were insufficient data to draw any conclusions. However, the observed efficacy of fish oil delivered by enteric coated capsule on reducing steroid requirements did warrant more attention (113).

Another systematic review and meta-analyses by Turner et al. (101) looked at the efficacy and safety of n-3 fatty acid or fish oil therapy in maintaining remission in inflammatory bowel disease. Of the nine studies eligible for inclusion, only three involved ulcerative colitis. There was no difference in the relapse rate between the n-3 fatty acid therapy (fish

oil) and control groups. Pooled analysis showed an increase in diarrhea and symptoms of the upper gastrointestinal tract in the n-3 fatty acid group (fish oil) suggesting troublesome side effects. In short, there was insufficient data to recommend the use of n-3 fatty acids for the maintenance of remission of ulcerative colitis. Given the biologic rationale and the benefit of n-3 fatty acid therapy derived from tissue samples and animal models, it is difficult to explain the lack of clinical benefit in inflammatory bowel disease, although it has been suggested that the dosing regimen may be inadequate or the formulation not optimal (101). Enterically coated n-3 fatty acid that has a timed release of 60 minutes upon ingestion was found to be more beneficial with the lowest adverse events compared with other timed release points and triglyceride compounds (130). In conclusion, further studies are warranted to address appropriate dosing and delivery systems of fish oil for the treatment of ulcerative colitis.

8.2 Plant derived therapies

Other novel therapies that possess anti-oxidant, anti-inflammatory and immunomodulatory properties have been investigated in experimentally-induced animal colitis models and to some extent in human trials for the treatment of ulcerative colitis. Persistent ulcerative colitis is associated with a 10-fold increased risk of colorectal cancer (131) and therefore limiting chronic colonic inflammation will appear to reduce this risk.

Resveratrol

Resveratrol is a natural polyphenol found in fruits and vegetables and abundantly in grapes and red wine. Sanchez-Fidalgo et al. (132) investigated the protective/preventive effects of dietary resveratrol in the DSS-induced colitis mouse model. There were significant attenuations of clinical signs of colitis such as loss of body weight, diarrhea and rectal bleeding. All mice fed the resveratrol diet survived and finished the treatment while mice fed the standard diet showed a 40% mortality rate. Resveratrol caused significant reductions in TNF α and IL1 β and an increase in IL10, an anti-inflammatory cytokine. Expression of prostaglandin E synthase-1 (PGES-1), cyclooxygenase (COX-2) and iNOS, proteins involved in the inflammatory response, were also reduced. Cui et al. (133) investigated the protective/preventive effects as well as the chemopreventive properties of dietary resveratrol in the chronic DSS-induced colitis mouse model. Resveratrol was shown to ameliorate colitis in a dose dependant manner and reduce the tumour incidence by 60%. The number of tumours per animal was also reduced. Resveratrol is tolerated at high doses and a diet rich in this polyphenol could represent a novel approach to treating ulcerative colitis and preventing colon cancer associated with ulcerative colitis.

Andrographis paniculata

Andrographis paniculata, a member of the plant family Acanthaceae, is used extensively in Asian countries, Sweden and Chile for the treatment of various inflammatory and infectious diseases. HMPL-004, an aqueous ethanol herbal extract of Andrographis paniculata has been shown to inhibit TNF α and IL1 β and prevent colitis in animal models. A pilot human clinical trial conducted by Tang et al. (134) investigated the efficacy and safety of HMPL-004 in patients with mild to moderate ulcerative colitis. In comparison to a parallel group treated with the standard first line therapy, mesalazine, there were no significant differences observed for clinical remission and disease activity. 13% of patients treated with HMPL-004 and 27% treated with mesalazine had at least one adverse event although the majority of

events were not strongly linked to the study medications. In conclusion, HMPL-004 could be an efficacious alternative to mesalazine for the treatment of ulcerative colitis.

Black raspberries

As well as exhibiting ability to limit the inflammatory response in cell culture (135-137), black raspberries (BRB) have the highest concentration of antioxidant polyphenols compared to other dark berries (138-139). These antioxidants (anthocyanins and ellagic acid) have been shown to scavenge free radicals, increase expression of detoxification enzymes and increase the capacity of the cell to absorb radicals (140-143). A study conducted by Montrose et al. (144), the first to utilise a DSS-induced mouse model of ulcerative colitis to explore the effects of freeze-dried BRB on disease severity, demonstrated the high anti-inflammatory potency of BRB. Dietary BRB markedly reduced colonic injury to the epithelium and tissue levels of TNF α and IL1 β were suppressed. Biomarkers of oxidative stress remained unaffected by BRB treatment, however the findings still demonstrated potent anti-inflammatory properties which support a possible therapeutic role for the treatment of ulcerative colitis.

American ginseng

American ginseng (AG), a natural herb, has been shown to improve mental performance and end points associated with conditions such as cardiovascular disease, cancer and diabetes (145-147). In a study by Jin et al. (148), AG extract was mixed in with the chow of DSS-induced colitic mice and given before and after the onset of colitis. Results showed prevention and treatment of colitis with AG along with the downregulation of iNOS and COX-2 and p53 (induced by inflammatory stress). In part, leukocyte activation in colitis causes mucosal and DNA damage which was shown to be inhibited by AG *in vitro* and *in vivo*. A dysfunctional intestinal immune system is a major mechanism by which chronic inflammation occurs in ulcerative colitis and defects in apoptosis of mucosal inflammatory cells is critical in the pathogenesis of ulcerative colitis. Another study conducted by Jin et al. (149), showed that AG extract can drive apoptosis of inflammatory cells through the p53 mechanism *in vitro* which is consistent with dietary AG protecting against DSS-induced colitis in the mouse model.

Ginkgo biloba

Ginkgo biloba extract (EGB) is derived from the green leaves of the Ginkgo biloba tree and has been used extensively in conditions associated with inflammatory mediators such as acute pancreatitis, central neural system disorders, heart and intestine injury/reperfusion injury (150-152). Zhou et al. (153) investigated the mechanism by which EGB ameliorates inflammation in TNBS-induced colitic rats and its effects on the production of inflammatory mediators. Four weeks of EGB therapy provided protection in ulcerative colitis possibly by radical scavenging and down regulating some of the inflammatory mediators including TNF α , NF κ Bp65 and IL6. All inflammatory mediators in this study were affected by EGB in a dose dependant manner resulting in the improvement of ulcerative colitis. Another study by Kotakadi et al (154) showed that EGBs have anti-inflammatory properties *in vitro* and prevent and treat colitis in the DSS-induced mouse model. The mechanism underlying the treatment of ulcerative colitis is in part due to the ability of EGB to drive CD4⁺ effector T cell apoptosis which is fundamental in regulating many chronic inflammatory and autoimmune diseases (155-156).

8.3 Other potential therapies

Crocin

Crocin, a carotenoid compound derived from *Crocus sativus L* (saffron) has been used to treat different diseases (157). In the TNBS-induced colitis mouse model, it was revealed that treatment with 50 mg/kg/day intragastrically for 8 days significantly ameliorated diarrhea, inflammation and colonic tissue injury (158). The mechanisms by which crocin exerted these beneficial effects is through the reduction of neutrophil infiltration and MDA in the inflamed colon. Increased production of NO by iNOS and activation of NF κ B, known to play a central role in the early steps of inflammation were also reduced. With further investigation, crocin could prove to be an alternative therapy or perhaps be used alongside conventional therapies.

Pomegranate

Punica granatum or the pomegranate is used in traditional medicine in China, India, Europe and South Africa. Studies have shown that pomegranate has protective properties against liver fibrosis and ultraviolet-induced pigmentation (159-160). Furthermore, it has antibacterial, anti-inflammatory, anti-diabetic effects and is cardio-protective (161-163). Singh et al. (164) explored the effect of *Punica granatum* extract and its component, ellagic acid, in the DSS-induced colitis mouse model and found significant attenuation of colonic inflammation. Mast cell degranulation, which releases various inflammatory mediators, including histamine, has been implicated in the pathogenesis of ulcerative colitis and the use of mast cell stabilizers have been documented to attenuate the severity of ulcerative colitis in humans (165). Singh et al (164) found that *Punica granatum* extract and its ellagic acid component had anti-ulcerative effects comparable to sodium cromoglycate (mast cell stabiliser) and sulphasalazine (standard first line treatment for ulcerative colitis).

Helminths

Immune-mediated diseases such as inflammatory bowel disease are becoming more prevalent in highly developed and industrialized countries (166). It is suggested that the adoption of hygienic lifestyles in these countries have contributed to a decline in helminths or parasitic worm infections (166). Epidemiological studies (167-169) have suggested that helminths may provide protection against some immune-mediated diseases and the eradication may in fact promote these diseases. Animal studies have shown helminth protection by promoting regulatory immune responses. In a DNBS-induced mouse colitis model Melon et al. (170) showed that mice infected with the tapeworm, *Hymenolepis diminuta*, increased the production of IL4 and IL10 that protected them from colitis, in contrast to steroid treatment (dexamethasone) which offered little benefit. Khan et al. (171) also showed protection with the nematode, *Trichinella spiralis*, in the TNBS- induced colitis mouse model. However, it was found that helminth infection enhanced disease severity in the oxazolone induced colitis mouse model (172). In a human randomized crossover trial conducted by Summers et al (173), a significant percentage of patients with ulcerative colitis receiving the porcine whipworm, *Trichuris suis*, improved when compared to placebo. As well, the treatment seemed to be safe with no reported side effects. In conclusion, there is potential value for helminth therapy for specific inflammatory bowel disease patients however further studies are needed to fully understand the mechanisms underlying the pathophysiology of ulcerative colitis and the type of helminth therapy required to avoid the possibility of disease aggravation.

8.4 Prebiotics

There is a diverse and large population of micro-organisms naturally living on the mucosal surfaces or in the lumen of the human intestine. The number of resident bacteria increases along the small bowel, with the colon being the most heavily populated region of intestine. The microbiota refers to the particular ecological niche of a host individual in which the community of living micro-organisms is assembled (174). A healthy or balanced microbiota has been considered to be predominantly saccharolytic and comprises of significant numbers of lactobacilli and bifidobacteria (175). A prebiotic can be defined as a non-digestible food ingredient that exerts a beneficial effect on the host through the selective stimulation and metabolism in the intestine, thereby improving host health (176). Inulin and oligofructose are prebiotic carbohydrates that resist digestion by intestinal and pancreatic enzymes in the human gastrointestinal tract and are fermented by bacteria living in the intestinal ecosystem. Prebiotics increase saccharolytic activity within the gut and selectively promote the growth of bifidobacteria when administered in significant amounts (177-178).

Animal models

Videla et al. (179) investigated the effectiveness of inulin, which stimulates intracolonic generation of butyrate and growth of lactic acid bacteria, in the protection against colitis. In a rat model of DSS-induced colitis, oral inulin treatment significantly reduced colonic tissue MPO activity and mucosal release of inflammatory mediators (179). Histologically, oral inulin treatment reduced the extent of damaged mucosa, decreased the severity of crypt disruption and lowered histological damage severity scores compared with controls. Inulin induced an acidic environment from the caecum to the left colon and increased counts of Lactobacilli (179).

Fructooligosaccharides (FOS) increase the growth of lactic acid bacteria and promote butyrate and lactate production, therefore possessing beneficial properties for intestinal inflammation (180). Intracolonic TNBS-induced colitic rats treated with intragastric infusions of FOS resulted in a reduction of pH and inflammation assessed by MPO activity. Furthermore, FOS treatment increased lactate and butyrate concentrations including lactic acid bacteria counts in the caecum (180).

Madsen et al. (181) investigated the role of colonic aerobic luminal bacteria and lactobacillus species in IL10 gene-deficient mice that spontaneously develop colitis. These knockout mice have a decreased level of lactobacillus species in the colon and an increase in adherent and translocated bacteria in the neonatal period. Normalising Lactobacillus levels via oral lactulose therapy reduced colonic mucosal bacteria and prevented colitis (181). Similarly, lactulose treatment has demonstrated protective effects against DSS- and TNBS-models of colitis (182-183). Rumi et al. (182) demonstrated that lactulose therapy ameliorated DSS-induced colitis in a dose-dependent manner and significantly reduced the severity of colonic lesions and decreased MPO activity. Furthermore, Camuesco et al. (183) indicated that lactulose treatment in TNBS-induced colitis exerted a preventive anti-inflammatory effect as evidenced by a significant reduction of MPO activity, a decrease of colonic TNF α and leukotriene B4 production and an inhibition of colonic inducible nitric oxygen synthase expression, which is a result of the inflammatory process (183). Furthermore, this effect was associated with increased levels of lactobacilli and bifidoacteria species in colonic content when compared with untreated colitic rats (183). Overall, the experimental evidence provides significant indications of the anti-inflammatory and beneficial properties of prebiotics in settings of ulcerative colitis.

Clinical studies

Recently, Casellas et al. (184) tested the effect of oligofructose-enriched inulin, which promote the selective growth of saccharolytic bacteria with low inflammatory potential, in patients with active ulcerative colitis. Eligible patients in the randomized, placebo-controlled double blinded pilot trial had been previously in remission with mesalazine as maintenance therapy or no drug, and presented with a relapse of mild to moderate activity (184). Nineteen subjects were treated with mesalazine and randomly allocated to receive either oligofructose-enriched inulin or placebo for two weeks. Patients treated with oligofructose-enriched inulin displayed a reduction of faecal calprotectin, a protein found in granulocytes that resist metabolic degradation (184).

8.5 Probiotics

Probiotics are defined as living, non-pathogenic bacteria which are able to exert beneficial therapeutic or physiologic activities when administered in sufficient numbers (185). Bacteria can be derived from various sources such as cultured food and the normal human microbiota. *Lactobacillus* or *bifidobacterium* genera are the most common strains of probiotic bacteria and have also been identified from enterococcus, streptococcus, and lactococcus species, while certain non-pathogenic *Escherichia* strains are also classified as probiotics (186). Furthermore, genetic engineering of probiotic strains can ensure the release of bioactive compounds¹⁶. The beneficial effects of probiotics are highly species and strain specific and therefore the mechanism of action is not well understood. Common mechanisms of action identified in probiotics include improvement of epithelial barrier function, inhibition of pathogenic enteric bacteria and manipulation of host immunoregulation (185).

In vitro models

The effects of probiotics have been investigated using recent comprehensive cell culture experiments which are model systems of inflammation and infection similar to ulcerative colitis. Schlee et al. (187) investigated the ability and mechanism by which different probiotic *Lactobacillus* strains, including *L. Acidophilus* PZ1138, *L. Fermentum* PZ-1138, *E. coli Nissle 1917* and VSL#3 (a combination of 8 bacterial strains), stabilize gut barrier function via induction of the anti-microbial peptide human beta defensin-2 (hBD2) gene. The expression of hBD2 gene by probiotic bacteria was both time- and dosage-dependent, and the promoter activation by probiotics was completely inhibited via deletion of NFκB and activator protein-1 (AP1) binding sites on the hBD-2 promoter (187). Furthermore, hBD-2 induction was also hindered by the inhibition of mitogen-activated protein kinase (MAPK). Overall, Schlee et al. (187) demonstrated that *Lactobacilli* and the VSL#3 bacterial combination strengthened intestinal barrier functions via the up-regulation of hBD-2 through induction of MAPKs and pro-inflammatory pathways including NFκB and AP1. In support of the finding of Schlee et al. (187), *E. coli Nissle 1917* was further demonstrated to strengthen intestinal barrier function using a polarized T84 epithelial monolayer model to monitor barrier disruption by *E. coli* infection (188). Co-incubation of the enteropathogenic *E. coli* strain with *E. coli Nissle 1917* or addition of *E. coli Nissle 1917* following infection abolished barrier disruption and restored barrier integrity (188). DNA-microarray analysis of T84 cells incubated with the enteropathogenic *E. coli* identified altered expression of over 300 genes, including the distribution of zonula occludens-2 (ZO-2) protein and of distinct

protein kinase C isotypes, all of which are involved in the maintenance of epithelial tight junctions (188).

Furthermore, *E. coli Nissle 1917* has been shown to exert anti-inflammatory effects on human colonic epithelial cells *in vitro* (189). Enzyme-linked immunosorbent assays and real-time quantitative PCR demonstrated that *E. coli Nissle 1917* treatment *in vitro* suppressed TNF α -induced IL8 transcription and production and inhibited IL8 promoter activity. These properties, in conjunction with the hBD2 results from Schlee et al (187) and T84 epithelial monolayer model results from Zyrek et al. (188) contribute to the reported efficacy in the treatment of inflammatory bowel diseases. Due to the unfortunate idiopathic nature of IBD, pre-treatment with probiotics may be more beneficial for either genetically susceptible individuals or to help IBD sufferers maintain remission.

Animal models

Several murine models of intestinal damage have been utilised to assess the efficacy of probiotics *in vivo* (190-192). Ukena et al. (191) orally administered the probiotic *E. coli Nissle 1917* to BALB/c mice with acute dextran sulphate sodium (DSS)-induced colitis. The probiotic treatment resulted in an upregulation of the tight junction molecule ZO-1 in intestinal epithelial cells at both mRNA and protein levels and reduced intestinal barrier permeability (191). Additionally, infiltration of the colon with leukocytes was ameliorated in *E. coli Nissle 1917* inoculated mice (191). Furthermore, Grabig et al (193) demonstrated that *E. coli Nissle 1917* treatment in a wildtype DSS-induced colitis mouse model significantly reduced pro-inflammatory cytokine expression, myeloperoxidase (found in the intracellular granules of neutrophils) activity and disease activity. The inability of *E. coli Nissle 1917* to exert its beneficial effect in the absence of toll-like receptor (TLR)-2 and TLR4 signaling using TLR2 and TLR4 knockout mice indicates that the amelioration of experimentally-induced colitis in mice was elicited via TLR2- and TLR4-dependent pathways (193). This finding highlights the fact that *E. coli Nissle 1917* may improve the ability of TLRs, which are key components of the innate immune system that trigger antimicrobial host defence responses, to recognise microbial pathogens, improving the host immune response.

Lee et al. (194) demonstrated that oral *L. plantarum HY115* treatment to mice with DSS-induced colitis inhibited colon shortening and MPO production. Furthermore, *L. plantarum HY115* repressed the mRNA expressions of IL1 β , TNF α and IFN γ , including colonic IL1 beta and IL6 protein expression and reduced the degradation activities of chondroitin sulphate and hyaluronic acid of intestinal bacteria (194). Similarly, Schultz et al. (195) immune-mediated colitic (induced by IL10 deficiency) mice treated with *L. plantarum* had decreased levels of mucosal IL12, IFN γ and immunoglobulin G2a (195).

A study by Peran et al. (196) assessed the intestinal anti-inflammatory effects of probiotics with immunomodulatory properties in the TNBS rat model of colitis. *L. casei*, *L. acidophilus* and bifidobacterium lactis elicited intestinal anti-inflammatory effects, evidenced macroscopically by a decreased colonic weight/length ratio and biochemically, all probiotics restored colonic glutathione levels, depleted due to oxidative stress (196). Interestingly, each probiotic displayed a unique anti-inflammatory profile; bifidobacterium lactis reduced colonic TNF α production, *L. casei* decreased colonic COX-2 expression and *L. acidophilus* reduced leukotriene B4 production and MPO activity (196). These findings indicate that probiotics exert their beneficial effects via different mechanisms. Menard et al. (197) inoculated gnotobiotic mice with bifidobacterium longum NCC2705 and nine bifidobacterium strains isolated from infants' faecal flora to investigate the effect of these

probiotics on the Th1/Th2 balance. Immunomodulatory responses including induction of the Th1 and Th2 cytokines, increased ileal IL10, IL4, TNF α and IFN γ secretions and TGF β 1 gene expressions, were observed from only specific strains (197). It was concluded that bifidobacterium's capacity to stimulate immunity is species specific however its influence on the orientation of the immune system is strain specific.

Clinical trials

To date, probiotics have been investigated in several clinical trials as treatments for ulcerative colitis, with conflicting results. However, there have been relatively few large, placebo-controlled, randomised and double-blinded clinical studies to test the efficacy of probiotics in humans (198). Tsuda et al. (199) evaluated the efficacy of the probiotics combination therapy BIO-THREE, comprising of *Streptococcus faecalis* T-110, *Clostridium butyricum* TO-A and *Bacillus mesentericus* TO-A, in patients with mild to moderate distal ulcerative colitis. Patients ingested nine BIO-THREE tablets per day for four weeks. Clinical symptoms and endoscopic findings were evaluated as ulcerative colitis disease activity index and faecal samples were collected to assess the microflora, pre- and post-treatment (199). Remission was observed in nine patients (45%), response in two patients (10%), no response in eight patients (40%) and worsening in one patient (5%) (199). Interestingly, terminal restriction fragment length polymorphism (T-RFLP) analysis indicated that the principal alteration in microflora was an increase in bifidobacteria (199); an unusual finding as no bifidobacteria was administered in the probiotic supplement.

8.6 Zinc

Zinc is ubiquitous in biologic systems and has abundant and varied functions. The zinc atom has the ability to participate in readily exchangeable ligand binding in addition to assuming a number of coordination geometries to provide functional needs to other ligands (200). Zinc has numerous central roles in DNA and RNA metabolism (201). Zinc metalloenzymes and zinc-dependent enzymes have been identified and are involved in nucleic acid metabolism and cellular proliferation, differentiation and growth (202). Zinc also plays a regulatory role in apoptosis (203), with cytoprotective functions that suppress major pathways, leading to programmed cell death.

Animal studies

Zinc administration has been shown to suppress the development of DSS-induced colitis in mice as indicated by decreased clinical disease activity index and histological severity scores (204-206). Ohkawara et al. (205) demonstrated that polaprezinc (N-(3-Aminopropionyl)-L-histidinato zinc), an anti-ulcer drug, suppresses DSS-induced colitis in mice, partly through inhibition of production of pro-inflammatory cytokines, suppression of neutrophils accumulation and cytoprotection by overexpression of heat shock proteins. This is consistent with Iwaya et al (207) whom reported that marginal zinc deficiency exacerbated colitis by modulating the immune response through the impairment of TNF α production and TNFR1 expression, rather than through the impairment of epithelial barrier function. Another potential mechanism of action of zinc in ulcerative colitis has been suggested by Luk et al. (208) by reducing inflammation, inhibiting mast cell degranulation and histamine release. In addition, high dose of zinc has been shown to improve tight-junction permeability (209). A novel zinc compound, Z-103, a chelate compound consisting of zinc ion and L-carnosine, was utilized to assess the protective effect against colonic damage

induced by TNBS in rats (210). The authors demonstrated that treatment with Z-103 reduced the inflammatory responses induced by TNBS, suggesting Z-103 may be as effective against TNBS-induced colitis.

Metallothioneins (MTs) are zinc-binding proteins whose overexpression may lead to sequestration of zinc ions. We have shown that the absence of MT was beneficial in the suppression of colitis in MT knockout (MT^{-/-}) mice receiving DSS, suggesting that the presence of MT may have promoted the induction of colitis. Similarly, as indicated by the histological severity scores, MT wildtype mice appeared more susceptible to DSS-induced colitis compared to MT^{-/-} animals (204). Furthermore, Bruewer et al. (211) reported that MT overexpression may represent an important early step in the development of carcinogenesis of ulcerative colitis independent of p53 expression. This should be further investigated in the long term as an independent cancer risk factor in ulcerative colitis.

Human studies

The only double-blind controlled trial of oral zinc sulphate as adjuvant treatment in idiopathic ulcerative colitis or proctitis in relapse was reported by Dronfield et al. (212). In this trial, 51 patients were treated with zinc and the clinical and sigmoidoscopic improvement was similar in the treated and placebo group. Furthermore, it has been shown that zinc administration decreased peripheral blood natural killer cell activity in 13 inflammatory bowel disease patients, with stable disease and mild-moderate disease activity, in a double-blind randomized cross-over trial (213).

9. Conclusions

Animal models of acute and chronic intestinal inflammation are indispensable for our understanding of the pathogenesis of ulcerative colitis and Crohn's disease, even though the etiology of inflammatory bowel disease remains unclear. In conclusion, administration of the above novel therapies have potential benefits in suppressing clinical features, histological pathology scores and inflammatory indicators in colitis in experimental models. There are four types of experimental animal models of colitis; spontaneous colitis models, inducible colitis models with normal immune system, adoptive transfer models in immunocompromised hosts, and genetically engineered models (knockout and transgenic mice). There is not one single experimental model of colitis that incorporates all the clinical and histopathological characteristics of human inflammatory bowel disease, however, information gained from studies using these different types of colitis models has revealed three fundamental underlying principles. Firstly, chronic intestinal inflammation is mainly mediated by T cells. Secondly, commensal enteric bacteria are required to initiate and achieve intestinal inflammation and finally, the genetic background of the animal is a pivotal factor of disease onset and severity (84). Using these different models of colitis, *in vitro* and *in vivo* studies have shown a variety of novel therapies including, pre- and probiotics, n-3 fatty acids, plant bioactives (resveratrol, black raspberries, ginseng, ginkgo) and helminthes which have potential benefits in suppressing clinical features, histological pathology scores and inflammatory indicators. These novel therapies act on specific mechanisms of action such as intestinal barrier function, mucosal immune function and intestinal microbiota, however, there are no single therapies that have a multifunctional mechanism of action to prevent and treat ulcerative colitis. Newer therapies which use a combination of agents to restore gut homeostasis should be more promising and closer to

achieving long-term remission of ulcerative colitis. Thus, further studies are warranted to determine the mechanism of action by which these agents are able to protect against ulcerative colitis and to explore whether combination therapy could produce synergistic effects.

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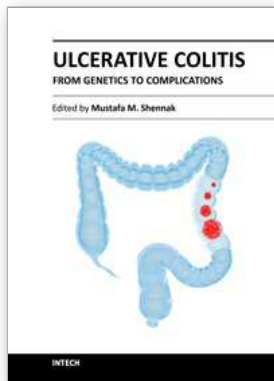
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Ulcerative Colitis (UC) is a rapidly evolving medical field, and will continue to be very exiting in the next few decades. Although the underlying cause of this disease is still unknown, results in research dealing with various issues related to this disease are published every day. Chapters included in this book review the most recent literature on related advancements in regard to this chronic disease, which is controllable but not curable. Aspects like epidemiology, pathophysiology, genetics, incriminated etiologies, clinical aspects, complications, and disease management, including advancements in the diagnostic and therapeutic options, were documented by well known clinicians, researchers, and world wide authorities in their fields. This book on UC will be a valuable addition to each doctor's library interested in this subject, or for physicians dealing with patients suffering from this disease. Authors have also included figures and diagrams to depict their point, and to easily reach the minds of the readers in the simplest way.

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