Abstract

The pathogenesis of Crohn’s disease likely involves multifactorial interactions between genetic factors and environmental triggers. The most recent studies suggest that luminal bacteria are a significant factor in the onset and chronicity of inflammation. In interleukin-10 (IL-10) gene-deficient mice a Crohn’s-like colitis develops when the mice are raised under conventional animal care facilities but fails to develop when they are raised under germ-free conditions. These mice demonstrate significant alterations in the species and the levels of bacteria colonizing the colon, suggesting that genetic factors in the host may be critical in controlling bacterial colonization. In addition, early treatment of IL-10 gene-deficient mice with antibiotics can prevent the development of colitis in later life, suggesting that early events during the neonatal period can influence later disease progression. Recent work has focused on using probiotic bacterial mixtures to alter the microbial balance in the colon in attempts to reduce inflammation. The use of the VSL-3 probiotic mixture in the IL-10 gene-deficient mouse resulted in a complete normalization of physiological transport function and barrier integrity, in conjunction with a reduction in mucosal secretion of TNF-α and IFN-γ. Further, it would appear that a soluble factor is released from a bacterium found in the VSL-3 mixture that can act directly on the epithelium to enhance barrier function.

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Medical subject headings: bacteria; Bifidobacterium; colitis; Crohn disease; genes; inflammatory bowel diseases; interleukin-10; Lactobacillus; mice; probiotics; research


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The Joe Doupe Young Investigators’ Award

In 2000, the contributions made by Dr. Karen L. Madsen were recognized by the Canadian Society for Clinical Investigation (CSCI) with the Joe Doupe Young Investigators’ Award. As is usual, the presentation was made at the annual meeting of the CSCI and the Royal College of Physicians and Surgeons of Canada in the year following Dr Madsen’s acceptance address on inflammatory bowel disease.

Dr. Joe Doupe was head of the Department of Physiology at the University of Manitoba for 18 years. During his time there, he founded a Bachelor of Science in Medicine course. For those who did not know Dr. Doupe, it is difficult to appreciate how many excellent scientists came through this course to subsequently make their mark in the scientific world. Dr. Arnold Naimark, who knew Dr. Doupe well, wrote, “He showed his students that, in the pursuit of true scholarship, no asset is more telling than independence of mind. It brooks no limitations. He demonstrated that independence of mind is the guarantee of the scholar’s security from the tyranny of transient fashions in medicine, and allows reason to rise above passion. With independence of mind the student has the freedom to seek and find his own destiny; without it he must accept the fate of others.”

This award was established by the CSCI to recognize excellence in research training in the initial stages of independent investigation. It is an award for performance and promise, qualities that Dr. Doupe recognized, nurtured and prized.

Inflammatory bowel disease: lessons from the IL-10 gene-deficient mouse

Clin Invest Med • Vol 24, n° 5, octobre 2001
Inflammatory bowel disease

Introduction

Despite many years of extensive research, the pathogenesis of Crohn’s disease remains unclear. Recent advances support multifactorial interactions between genetic factors and environmental triggers, as the incidence of Crohn’s disease is increasing markedly in the West. The most recent studies indicate that luminal bacteria are a significant factor in the onset and chronicity of inflammation. Indeed, the colon is in continual contact with numerous different bacterial species ($10^{10}$–$10^{12}$ colony forming units [CFU]/g tissue) and must continually define selective action toward nonpathogenic and pathogenic components. Under normal conditions, mucosal tolerance exists toward the body’s own nonpathogenic bacteria, that is, an immune response is not mounted against such bacteria. The current hypothesis for the development of Crohn’s disease is that an abnormal immune response to the body’s own normal microflora occurs owing to a loss of tolerance. This loss may result from a dysregulation of immune mediators or a breakdown in barrier function, allowing a constant access of inflammatory bacterial products to the resident immune system and thereby overwhelming normal downregulation. Thus, the search for environmental triggers has recently focused on bacterial and viral organisms, and possible alterations in the microflora colonizing the colon due to dramatic changes that have occurred over the last century in food production and consumption.

Microflora and Crohn’s disease

Evidence from numerous murine models indicates that any disruption of normal mucosal homeostasis, including alterations in cytokine production, production of regulatory immune cells or loss of barrier integrity can lead to intestinal inflammation. An interesting finding, however, with all of these models, is that when these animals are raised under germ-free conditions, there is either attenuated or no in-

Résumé

La pathogénèse de la maladie de Crohn met probablement en cause des interactions multifactorielles entre des facteurs génétiques et des déclencheurs environnementaux. Les études les plus récentes indiquent que les bactéries luminales jouent un rôle important dans l’apparition et la chronicité de l’inflammation. Dans des souris qui ont une déficience du gène *interleukine-10* (*IL-10*), une colite semblable à la maladie de Crohn fait son apparition lorsque les souris sont élevées dans des installations classiques de soin des animaux, mais non lorsqu’elles sont élevées dans un contexte aseptique. Ces souris montrent des modifications importantes des espèces et des niveaux de bactéries qui colonisent le côlon, ce qui indique que des facteurs génétiques de l’hôte peuvent jouer un rôle critique dans le contrôle de la colonisation bactérienne. En outre, le traitement rapide aux antibiotiques des souris qui ont une déficience du gène *IL-10* peut éviter l’apparition ultérieure de la colite, ce qui indique que des événements qui surviennent tôt au début de la période néonatale peuvent avoir des répercussions sur l’évolution ultérieure de la maladie. Des travaux récents ont visé avant tout à utiliser des mélanges bactériens probiotiques pour modifier l’équilibre microbien dans le côlon afin d’essayer de réduire l’inflammation. L’utilisation du mélange probiotique VSL-3 chez la souris qui a une déficience du gène *IL-10* a normalisé complètement la fonction de transport physiologique et l’intégrité de la barrière, et réduit simultanément la sécrétion de TNF-α et d’IFN-γ par la muqueuse. Il semblerait en outre qu’une bactérie que l’on trouve dans le mélange VSL-3 libère un facteur soluble qui peut agir directement sur l’ épithélium afin d’améliorer l’intégrité de la barrière. Les résultats tirés de modèles animaux de la maladie intestinale inflammatoire indiquent que des hôtes vulnérables sur le plan génétique peuvent produire une réaction immunitaire cellulaire pathogène à certaines espèces bactériennes non pathogènes à cause d’un défaut de la tolérance immunologique et du manque de défenses appropriées dans la muqueuse. Des bactéries probiotiques semblent constituer une nouvelle solution prometteuse pour le traitement de problèmes cliniques associés à des altérations de la fonction barrière de l’intestin, y compris la maladie de Crohn.
intestinal inflammation.\textsuperscript{4,8,9} In that Crohn’s disease is known to occur at sites with the highest concentration of luminal bacteria, such as the colon and terminal ileum,\textsuperscript{10} this has led to the hypothesis that Crohn’s disease results from an immune response directed against intestinal microflora. However, a fundamental question remains: Is the intestinal flora aberrant in diseased patients or are we dealing with an overly aggressive immune response to the normal nonpathogenic resident flora?

Several studies have attempted to answer this question by examining the intestinal flora of patients with Crohn’s disease and comparing it with the bacteria found with normal control patients. Giaffer and associates\textsuperscript{11} showed that the intestinal flora of patients with active Crohn’s disease and ulcerative colitis was considerably different from that of patients with quiescent disease, ulcerative colitis or normal control patients. In patients with active inflammatory bowel disease (IBD), the concentration of aerobic bacteria, especially \textit{Escherichia coli}, was elevated, and within the fraction of anaerobic bacteria, \textit{Bacteroides fragilis} and \textit{Bacteroides vulgatus} levels were increased. Additionally, in all patients with Crohn’s disease, \textit{Bifidobacteria} concentrations were decreased. Similar findings of reduced luminal and mucosal adherent levels of \textit{Bifidobacterium} and \textit{Lactobacillus} in humans with IBD disease have been described.\textsuperscript{12,13} This would suggest that Crohn’s disease may be linked to “abnormal” bacterial colonization. A role for bacteria in the pathogenesis of Crohn’s disease is also supported by clinical findings whereby the disease improves when luminal bacterial concentrations are decreased by a variety of techniques, including antibiotics, bowel rest, decontamination and lavage.\textsuperscript{14-16}

**Microflora in the IL-10-deficient mouse model of colitis**

In mice that are deficient in the gene for interleukin-10 a Crohn’s disease-like chronic colitis develops spontaneously shortly after weaning when the animal is raised in conventional animal care facilities. However, colitis does not develop when the animal is raised under germ-free conditions.\textsuperscript{5,8} The colitis in IL-10-deficient mice is characterized by dysregulation of the immune system, in that regulatory T cells either fail to develop or are functionally impaired in the absence of \textit{IL-10}.\textsuperscript{6} This leads to an IL-12 and IFN-\gamma-directed excessive generation and activation of Th1 cells and resultant immunopathology (Fig. 1).

Like patients who have Crohn’s disease, patchy ulceration, transmural acute and chronic inflammation and epithelial hyperplasia develop in \textit{IL-10}-deficient mice, beginning shortly after weaning.\textsuperscript{5,17} We have shown that these mice also have significant alterations in the species and the levels of bacteria colonizing the colon compared with control mice raised in the same environment.\textsuperscript{17} Again, this is similar to what has been seen in patients with Crohn’s disease. This altered pattern of bacterial colonization is present within 24 hours of birth, suggesting that the genetic background of the host influences early bacterial colonization and that alterations in bacterial colonization precede the development of colitis. Importantly, \textit{IL-10}-deficient mice have reduced levels of probiotic bacteria (\textit{Lactobacillus}) compared with controls. Furthermore, treating \textit{IL-10}-deficient mice from birth with \textit{Lactobacillus} actually prevents the development of colitis.\textsuperscript{17} This would suggest that the alterations seen in bacterial colonization in patients with Crohn’s disease may be an important factor in either the onset or the chronicity of inflammation, and that genetic factors may be critical in controlling which bacterial species are able to colonize the gastrointestinal tract.

Further supporting a role for distinct microflora in either the onset or the perpetuation of inflammation are studies showing that in patients who have Crohn’s disease, decreasing intestinal bacteria concentrations with metronidazole,\textsuperscript{18} ciprofloxacin\textsuperscript{19} or carithromycin and rifabutin\textsuperscript{14} results in an improvement in the inflammatory condition. These studies are mirrored by results that we have obtained in the \textit{IL-10}-deficient mouse model. Treatment of mice with either neomycin/metronidazole or ciprofloxacin during the neonatal period prevented the development of colitis for up to 12 weeks after withdrawal of the antibiotics.\textsuperscript{20} These data suggest that early intervention involving the alteration of normal intestinal flora in a genetically determined model for Crohn’s disease can alter disease progression in later
life. Thus, alterations of colonic flora (either a particular bacterial species or a reduction in the total bacterial load) with antibiotics during a critical period early in life may be a useful tool in preventing disease in genetically susceptible individuals with a family history of IBDs.

**Use of probiotics in the treatment of Crohn’s disease**

The term “probiotic” dates to 1965 when Lilly and Stilwell used it to describe substances or organisms that contributed to intestinal microbial balance, primarily of farm animals. More recently, probiotics have been defined as “living organisms, which on ingestion in certain numbers, improve the health of the host beyond their inherent basic nutrition.” This definition emphasizes the significance of maintaining a sufficient population of live microorganisms in the gut and further indicates that benefits can include an improvement in microbial balance associated with other health effects, such as immunomodulation. The main probiotic preparations currently being investigated belong to a large group of bacteria designated as lactic acid bacteria. The genera of lactic acid bacteria consist of a heterogeneous group of gram-positive bacteria, whose main fermentation product from carbohydrate is lactate. The group comprises cocci (Streptococcus, Pediococcus) and rods (Lactobacillus and Bifidobacterium), which are either exclusively (homofermentative) or at least 50% (heterofermentative) lactate producers. Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus brevis, Streptococcus thermophilus and Bifidobacterium infantis are important components of human gastrointestinal microflora and are used as probiotics for oral bacteriotherapy.

![Diagram of immune system and cytokines](image-url)

**Fig. 1:** Microbial antigens interact first with cells of the innate immune system. This system in turn activates naïve T cells, shown here at Th0, and induces cytokines that affect their differentiation pathway. Interleukin-12 stimulates Th0 cells to differentiate into the Th1 subset of CD4+ cells, which produce IL-2, IFN-γ and TGF-β. In contrast, if the activated Th0 cell encounters interleukin-4, it differentiates into the Th2 subset that produces IL-4, IL-5, IL-6, IL-10 and IL-13. The 2 subsets reciprocally inhibit one another by the production of IL-10 by Th2 cells and of IFN-γ by Th1 cells. In IL-10-deficient mice, colitis is initiated by the presence of microbes and consists of an IL-12 and IFN-γ-directed excessive generation and activation of Th1 cells.
Although few trials have investigated the effects of probiotic bacteria on human IBD, the idea of fighting bacteria with bacteria is not new. In 1995, Malin and colleagues published the results of a trial studying immune responses, especially that of IgA, in patients with Crohn’s disease after oral bacteriotherapy with *Lactobacillus GG*. Malin’s group were able to demonstrate increased numbers of IgA-secreting cells reacting to β-lactoglobulin and casein, specifically in children with Crohn’s disease. They concluded that *Lactobacillus GG* may have the potential to promote the gut immunologic barrier.

Recently, a new high-potency probiotic preparation (VSL-3) has been marketed in Europe. It has a greatly enriched population of live bacteria (10^{11} viable cells/g) and 4 strains of lactobacilli (*Lactobacillus casei*, *L. plantarum*, *L. acidophilus* and *Lactobacillus delbruekii subsp bulgaricus*), 3 strains of bifidobacteria (*Bifidobacterium longum*, *Bifidobacterium breve* and *B. infantis*), and 1 strain of *Streptococcus* (*Streptococcus salivaris subsp thermophilus*). This preparation possesses 2 main innovative characteristics: a very high bacterial concentration and a mixture of different bacterial species (with potential synergistic associations) to enhance the suppression of potential pathogens. Clinical studies have shown that VSL-3 has significant clinical potential in the treatment of patients with IBD. Gionchetti and associates studied VSL-3 in the treatment of refractory pouchitis. Forty patients with pouchitis were included in this double-blind, placebo-controlled study. The probiotic preparation was given for 6 months after remission-inducing therapy with antibiotics. At the end of the 9-month observation period, recurrence rates were 15% (3 of 20) for probiotic-treated patients and 100% for the placebo-treated group.

The use of VSL-3 as a treatment option for Crohn’s disease is supported by our studies in the *IL-10* gene-deficient mouse model. Treatment of adult *IL-10* gene-deficient mice with VSL-3 for 4 weeks resulted in complete normalization of colonic physiological transport function and barrier integrity. This occurred in conjunction with a reduction in mucosal secretion of TNF-α and IFN-γ and a significant improvement in the histologic appearance of the disease.

### Mechanisms of action of probiotic bacteria

Probiotic bacterial strains, such as *Lactobacillus* and *Bifidobacterium*, have demonstrated a protective role in several animal models of IBD that develop bacterial-induced intestinal inflammation. Several mechanisms have been proposed by which probiotics may exert their beneficial activity, including:

- (a) competitive exclusion of bacterial adherence or translocation, or both;
- (b) release of bacteriocidine and lactic acid, which can inhibit the growth of pathogens;
- (c) production of butyric acid, which enhances the turnover of enterocytes;
- (d) antioxidative effects;
- (e) probiotic enhancement of barrier function by stimulation of mucus and sIgA production;
- (f) an enhancement of macromolecular degradation by the gut mucosa, which acts to reduce the antigen load;
- (g) a suppression of immune cell proliferation; and
- (h) an inhibition of epithelial cell nuclear factor kappa B (NF-κB) activation.

Recently, we have shown that one or more of the bacteria found in the VSL-3 mixture appear to release a soluble factor that acts to enhance intestinal barrier function, which could conceivably be another mechanism of protection. Colons from *IL-10* gene-deficient mice demonstrate increased permeability as measured by increased movement of mannitol both in vivo perfusion and in vitro in Ussing chambers. After 4 weeks of VSL-3 therapy, mannitol fluxes were completely normalized in *IL-10* gene-deficient mice. Interestingly, although physiological ion function was not affected in control mice by VSL-3 treatment, mannitol fluxes were reduced, suggesting that the species of microflora present in the colon can directly alter colonic epithelial permeability, even in the absence of any inflammation. This was confirmed in studies using the T84 human colonic epithelial cell line. The application of the VSL-3 compound directly to epithelial monolayers resulted in an enhancement of barrier function and protection from subsequent pathogenic bacterial invasion, suggesting that epithelial cells can respond to bacterial signals. This effect was also seen when conditioned medium from the VSL-3 mixture was applied to monolayers, suggesting that a soluble factor was released, which was acting on the epithelial cells. This factor was heat labile and was sensitive to
proteinase K treatment, suggesting that the factor may be a protein.29

Intestinal barrier function and Crohn’s disease

The lumen of the intestine contains bacteria, bacterial products and dietary antigens capable of initiating and sustaining inflammation. However, the normal intestinal epithelium provides a barrier relatively impermeable to these luminal constituents; antigen uptake occurs in a controlled fashion through specialized antigen transport mechanisms in the epithelium and in Peyer’s patches.42,43

Patients with Crohn’s disease have increased mucosal permeability, either as a primary defect or as an acquired defect secondary to intestinal inflammation.44 An intriguing possibility is that a genetic or an environmentally induced disorder of intestinal barrier function exists in patients with Crohn’s disease and that it is the defect in barrier function that is an important etiologic factor. Evidence in support of an intrinsic permeability defect is suggested by reports of increased intestinal permeability in a subset of asymptomatic family members of patients with active Crohn’s disease.45-47 Nevertheless, regardless of whether the permeability defect is intrinsic or acquired, it is likely that increased permeability plays at least a secondary permissive role in the exacerbation of intestinal inflammation in Crohn’s disease by allowing increased antigenic uptake and continuous stimulation of the mucosal immune system. In genetically susceptible individuals who are unable to down-regulate the immune response, self-perpetuating, chronic intestinal inflammation could result.

In the IL-10-deficient mouse, a breakdown of intestinal barrier function precedes the development of inflammation by 2 to 3 weeks.41 This breakdown is accompanied by increases in colonic secretion of TNF-α and IFN-γ and is associated with the presence of colonic microflora, since mice raised under germ-free conditions do not demonstrate any alterations in barrier function.8, 41

Conclusions

Collectively, these results in animal models of IBD coupled with concurrent findings in patients with IBD suggest that genetically susceptible hosts can mount a pathogenic cellular immune response to specific nonpathogenic bacterial species, as a consequence of defective immunologic tolerance and lack of appropriate mucosal defences. It is very likely that the elimination of some aggressive subsets of luminal bacteria, combined with repopulation using beneficial probiotic bacteria will lead to a selective blockade of proinflammatory cytokines and Th1 lymphocytes, while simultaneously enhancing anti-inflammatory and protective immune responses. Indeed, probiotic bacteria appear to constitute a promising new treatment for clinical conditions that are associated with altered gut barrier function, including Crohn’s disease. There are currently 5 randomized, double-blind placebo-controlled clinical trials on-going, designed to assess the efficacy of VSL-3 in the treatment of IBD. The results from these trials, combined with controlled experimental laboratory data, should soon provide us with some answers. Indeed, all preliminary evidence to date points to probiotic bacteria as having a role in the stabilization of the gut microflora and the ability to modulate immune responses. Thus, the possibility that probiotic bacteria could be used for counteracting immunologic dysfunction in conjunction with stabilizing the gut mucosal barrier is a very real and exciting possibility.

References


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