LEADING ARTICLE

Dysbiosis in inflammatory bowel disease
C P Tamboli, C Neut, P Desreumaux, J F Colombel

Abundant data have incriminated intestinal bacteria in the initiation and amplification stages of inflammatory bowel diseases. However, the precise role of intestinal bacteria remains elusive. One theory has suggested a breakdown in the balance between putative species of “protective” versus “harmful” intestinal bacteria—this concept has been termed “dysbiosis”. Arguments in support of this concept are discussed.

Abundant data have incriminated intestinal bacteria in the initiation and amplification stages of inflammatory bowel diseases (IBD). The role of the NOD2/CARD15 Crohn’s disease (CD) susceptibility gene in bacterial peptidoglycan recognition strengthens the links between enteric bacteria and mucosal inflammation. Despite these advances, the precise role of intestinal bacteria remains elusive. Non-mutually exclusive theories have included: an unidentified persistent pathogen; an abnormally permeable mucosal barrier leading to excessive bacterial translocation; an immune system abnormality of effector cell activation or insufficient regulatory cell activity in response to intestinal bacteria; or a breakdown in the balance between putative species of “protective” versus “harmful” intestinal bacteria—this concept has been termed “dysbiosis”. Here we will focus on arguments to support this concept.

EXPERIENCE FROM EXPERIMENTAL MODELS OF COLITIS

The presence of intestinal bacteria is essential in several animal models of colitis. These models exist on varied genetic backgrounds, including mice that are deficient in interleukin (IL)-10, T cell receptor alpha beta, T cell receptor alpha, and also CD3-epsilon transgenic mice or HLA-B27 transgenic rats. Successful probiotic strategies have not been limited to bacteria: helminthic parasites also induce immunomodulatory T cell responses in the host. Exposure to eggs of Schistosoma mansoni has been shown to attenuate excessive Th1-type inflammation in the trinitrobenzenesulfonic acid colitis mouse model. Schistosoma egg exposure diminished interferon gamma levels, enhanced IL-4 production and IL-10 mRNA expression, and protected these mice from lethal inflammation. Of great interest will be studies exploring which components of probiotic organisms are important disease modifiers and how these components interact.

“Probiotic therapies have attempted to modify disease expression by favourably altering bacterial composition, immune status, and inflammation.”

In summary, experimental animal models have greatly contributed to our understanding of IBD pathogenesis. They have shown us that certain organisms and strains may be protective.

Abbreviations: IBD, inflammatory bowel disease; CD, Crohn’s disease; IL, interleukin; PCR, polymerase chain reaction; UC, ulcerative colitis
DYSBIOSIS IN IBD

In IBD, intestinal microflora have been analysed repeatedly. Although methodologies and results may differ, some generalisations are possible (for an overview see Linskens and colleagues). Earlier studies utilised conventional faecal based or mucosal bacterial isolation and culture techniques. These have often shown increased concentrations of anaerobes, particularly Gram negative anaerobes, including Bacteroides species in CD,\(^{22-23}\) ulcerative colitis (UC),\(^{26-27}\) and pouchitis.\(^{29-30}\) The pouchitis studies found increased numbers of Clostridium perfringens and other species not found in controls. Other studies incriminate Enterobacteriaceae, especially Escherichia coli, in CD.\(^{25-30}\) Many studies have also noted reductions in presumably beneficial bacteria, such as Bifidobacteria species in CD,\(^{25-26}\) UC,\(^{23}\) and pouchitis.\(^{29-30}\)

Several points require emphasis regarding studies on the intestinal flora in IBD. Firstly, most studies have used conventional bacteriological techniques that are inadequate for complete enumeration of the intestinal flora. Only up to 30% of the total microflora can be recovered in this way, and so molecular techniques analysing bacterial 16S ribosomal RNA components may improve the overall detection rate.\(^{34}\) Molecular techniques include polymerase chain reaction (PCR), in situ hybridisation, flow cytometry, and DNA microarray/chip analysis. However, even the use of molecular probes may leave significant numbers of bacteria undetected.\(^{35}\) Secondly, many strains in CD do not belong to major phylogenetic groups represented in healthy individuals.\(^{36}\)

Even in healthy subjects, up to 75% of the total bacteria remain unclassified species.\(^{36-37}\) The possible contribution of these strains to IBD remains unknown. Thirdly, a distinction should be made between mucosal flora and faecal flora. The assumption that the dysregulated immune response of IBD is targeted towards mucosal associated flora.\(^{39}\) As in faecal culture studies, mucosal analyses in IBD have found increased concentrations of anaerobes,\(^{25-27}\) 31-41 E coli,\(^{25-31}\) and decreased concentrations of Bifidobacteria species,\(^{15-41}\) including in the neoterminal ileum following ileocolonic resections for CD.\(^{33}\) There is also evidence to suggest an overall increase in mucosal concentrations of other bacterial species. Schultz et al used 16S rRNA probes with in situ non-fluorescent hybridisation to demonstrate increased bacterial load in the rectal mucosal layer.\(^{42}\) Most of the patients studied had UC. Similarly, Swidsinski and colleagues demonstrated thick layers of adherent mucosal associated bacteria in both UC and CD. Higher bacterial concentrations were found in Crohn’s subjects. Sites in simultaneous culture, quantitative PCR, and fluorescent in situ hybridisation analysis, 50% of CD patients had either E coli or Bacteroides species as the predominant group. Kleessen and colleagues\(^{15}\) used 16S rRNA probes to demonstrate increased mucosal concentrations of many species in UC but particularly E coli and Bacteroides species in CD. It should be noted that mucosal studies using molecular techniques do not always concur: Swidsinski and colleagues\(^ {46}\) found overall mucosal bacterial concentrations to be higher in CD than UC and higher in the ileum than in the colon, whereas Kleessen and colleagues’ found the reverse. Neither Schultz and colleagues nor Swidsinski and colleagues\(^ {46}\) found bacterial colonisation in control specimens or any relationship between bacterial invasion and the degree of mucosal inflammation but Kleessen and colleagues\(^ {15}\) did demonstrate bacteria in control specimens and increased penetration in areas of inflammation. Patient selection and complex processing requirements may account for some of these discrepancies.

‘Certain intestinal strains may be overrepresented in IBD, both in proportion and in immune responsiveness towards them’

Also worth considering are data from serological studies. Specific antibodies and T cell subsets have been demonstrated in serum and intestinal tissues of IBD patients.\(^{26-27}\) Reactivity to bacterial antigens is not unexpected with increased mucosal permeability or intestinal inflammation, and thus does not by itself add weight to the argument for dysbiosis. In this issue of Gut, Furrie and colleagues describe their simultaneous quantitative analysis of immune responsiveness to over 35 intestinal bacterial isolates in IBD subjects [see page 91]. Significantly higher systemic antibody responses were mounted in UC towards Peptostreptococcus anaerobius, in parallel with higher recovery rates of this strain from the colonic mucosa. This analysis provides evidence that certain intestinal strains may be overrepresented in IBD, both in proportion and in immune responsiveness towards them. Similarly, Landers et al have described heterogeneity of immune responsiveness to selected bacterial antigens in a large cohort of CD patients.\(^ {47}\) The proportions of seroreactive patients varied towards four microbial antigens, and cluster analysis of the seroreactivity patterns defined distinct patient subgroups. Although speculative, this raises the question of whether such antibody profiles might represent important differences in the composition of the mucosal flora or dysbiosis.

Emerging therapies for IBD include probiotics and prebiotics. There is good animal data to support the beneficial effects of many commensal bacteria on immune function and mucosal integrity. However, there remain very few well designed randomised clinical trials of probiotics in IBD. The most positive results have been for pouchitis prevention with a mixture of strains, VSL#3 (Yovis; Sigma-Tau, Pomezia, Italy).\(^ {48}\) VSL#3 also prevents relapse of chronic pouchitis.\(^ {49}\) As mentioned earlier, dysbiosis has been proposed as a key feature of pouchitis.\(^ {24}\) Prebiotic therapy—manipulation of diet to promote growth of beneficial intestinal microflora—is becoming increasingly studied and could prove to be particularly useful in improving dysbiosis. Colonic growth of Bifidobacteria strains is promoted by regular consumption of certain indigestible carbohydrates such as fructooligosaccharides and inulin, which are found at high concentrations in specific vegetable foods.\(^ {50-51}\)

‘How some bacteria may exert an inflammatory effect and others a protective role in IBD is as yet uncertain’

How some bacteria may exert an inflammatory effect and others a protective role in IBD is as yet uncertain. Strains of Bacteroides and Clostridia species can produce enterotoxins and/or possess proteolytic properties that enhance mucosal permeability and bacterial uptake.\(^ {52-53}\) There may also be detrimental effects of sulphide producing bacterial species in UC\(^ {54-55}\) or pouchitis.\(^ {25}\) Hydrogen sulphide blocks epithelial cell utilisation of short chain fatty acids, the preferred nutrient source for colonocytes. However, one animal model has shown sulphide production to be unimportant in DSS induced colitis.\(^ {55}\) Other investigations are focusing on the role of peptidoglycans, lipopolysaccharides, and bacterial CpG DNA motifs.\(^ {56}\) Studies from our group have shown that adherent and invasive E coli often colonise ileal lesions of CD. These strains appear to have all of the virulence factors required to colonise intestinal mucosa, cross the epithelial barrier, interact with resident macrophages, and induce the synthesis of proinflammatory cytokines by infected epithelial cells and macrophages.\(^ {57-59}\)
"Is dysbiosis just a secondary phenomenon of IBD, or is it actually a cause of IBD?"

Assuming dysbiosis is indeed a key element in the pathogenesis of IBD, perhaps the most vexing question is: what is the origin of dysbiosis? It was proposed many years ago that determination of our intestinal flora may be partly under genetic control.\(^1\) Alterations in faecal flora have also been found among healthy relatives of patients with IBD,\(^2\) suggesting that this may be an important a priori genetic risk factor for developing IBD. However, close family members also share their environment and the relative contributions of genetics and environment to one’s intestinal flora makeup are unclear. Environmental factors thought to be influential in determining the type (and rate of establishment) of normal intestinal flora include: mode of childbirth, maternal intestinal and vaginal bacterial colonisation patterns, weaning practices, and local environmental variables (hospital bacteria, level of hygiene, etc). Most studies of neonates show that intestinal colonisation progresses rapidly after birth, although factors determining the ultimate composition are unclear. Another key question related to this issue is: at birth, although factors determining the ultimate composition of IBD, or is it actually a cause of IBD? In favour of the former are observations that the bacterial profile is stable throughout life suggesting that the bacterial profile is stable throughout life has been challenged by suggestions that a Western diet, modern infant nutrition, antibiotic use patterns, and public health measures may favour the growth of relatively aggressive resident bacteria at the expense of beneficial commensals.\(^3\) Future IBD research endeavours should focus on developing testable hypotheses for each of these proposed risk factors.

Authors’ affiliations
C P Tamboli, Division of Gastroenterology, Department of Internal Medicine, University of Iowa Hospitals and Clinics, Iowa City, Iowa, USA
C Neut, Laboratoire de Bactériologie, Faculté de Pharmacie, Lille and INSERM EPI0114, CHU Lille, France
P Desreumaux, J F Colombel, Service d’Hépatogastroenterologie, Hospital Huriez and INSERM EPI0114, CHU Lille, France

REFERENCES
GI SNAPSHOT

Self inflicted rectal ulcer: hearing is believing

Question
A 54 year old woman presented with haematochezia of two weeks duration. She gave no history of straining at defecation or taking non-steroidal anti-inflammatory drugs. Physical examination was unremarkable. A colonoscopic image, showing a shallow longitudinal ulcer involving the anterolateral wall of the lower rectum and anal canal, is depicted in fig 1. Biopsies showed non-specific inflammation without the presence of fibromuscular obliteration. What further information should be obtained to make a definitive diagnosis? What is the most likely diagnosis? See page 20 for answer

Figure 1 Colonoscopic image, showing a shallow longitudinal ulcer involving the anterolateral wall of the lower rectum and anal canal.