

ROLE OF INFLAMMATION / INFECTION IN CHRONIC IDIOPATHIC INFLAMMATORY BOWEL DISEASES (IBD)

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Introduction

The link between chronic inflammatory bowel diseases (Crohn's disease and Ulcerative colitis) and infections is complex and has clinical and scientific implications. Common and less common intestinal infections can mimic Crohn's disease and ulcerative colitis.(1) Enteric and non-enteric infections can be associated with onset or relapse of IBD.(2, 3) Infections can be responsible for major complications of IBD (abscess, sepsis...) and since long infections have been considered as an element involved in the aetiology of IBD.(4) While the relation between the infection and inflammation is clear in some complications of IBD and in association with onset or relapse, the possibility of an infectious aetiology (causing persisting inflammation) of IBD remains to be clarified. This concept has however a long history. In 1902 Weir treated a patient with ulcerative colitis (UC) with irrigation with potassium permanganate for presumed infection.(5) Treatment with sulfonamides (1938) and then antibiotics (penicillin 1946) re emphasized the possibility of a bacterial infection. In the classic 1913 paper by Dalziel, chronic "interstitial ileitis" was attributed to Johne's mycobacterial intestinal disease of cattle. Early aetiologic speculations for Crohn's disease (CD) included bacteria, viruses and impaired vascular and lymphatic circulation.(6) Candidate causative agents include aerobic bacteria (Shigella, E. coli strains, Salmonella, Campylobacter jejuni, Yersinia), Mycobacteria, anaerobic bacteria and viruses (measles virus, paramyxovirus...). None of these pathogens has been unequivocally identified as etiological agent, although some may be associated with a first attack of IBD.(4)

Yet, many epidemiological data still point towards infection as a possible aetiology. Poor sanitation in infancy, living in prisons, lack of antibiotic use and infections are known to protect against CD, while improved domestic hygiene is a risk factor.(7, 8) The most frequent lesions in CD are within the ileal-colonic junction, which is the first intestinal segment to harbour a significant and polymorphous bacterial flora. Antibiotic therapy has been shown to have some efficacy in decreasing disease symptoms and disease activity increases rapidly following reconnection of bowel loops after resection or after reperfusion of faecal material into the afferent segment of a double-loop ileostoma.(9-11) Patients show an increased mucosal secretion of IgG antibodies against commensal flora.(12) Some studies provide evidence for the existence of a transmissible agent in human CD tissues.(13) Finally, the majority of animal models of inflammatory bowel disease require the presence of an intestinal flora if disease symptoms are to manifest.(14)

Three major theories are currently under consideration for the explanation of the relation between infections and the aetiology of IBD, especially CD : a) the disease develops due to a reaction to a persistent intestinal infection; b) the disease is due to the existence of a defective mucosal barrier to luminal antigens; c) the disease results from a dysregulated host immune response to ubiquitous pathogens. In each of these theories, either pathogenic or resident luminal bacteria constantly stimulate the mucosal and systemic immune systems to perpetuate the inflammatory cascade. In the majority of the recently developed rodent models of spontaneous chronic intestinal inflammation, disease developed as consequence of immune manipulations, suggesting a central role for the immune system in the regulation of intestinal inflammation. It is noteworthy to mention that inflammation usually did not develop in these animals when maintained in germ-free conditions. This finding suggests that disease may develop as a result of a dysregulated immune system (susceptible host) and an inappropriate response to (components of) the (normal) bowel flora. A combination of a defective barrier

and a dysregulated immune response responsible for increased susceptibility is another possibility.

The normal intestinal flora

The “indigenous flora” that inhabits the gastrointestinal tract is composed of the “autochthonous” and the “allochthonous flora (transiently present)”.(15) Colonization of the digestive tract develops immediately after birth. The intestinal tract of an individual contains some 300 to 500 different species of bacteria, that during millennia have evolved and adapted to live in the human intestinal habitat. The composition of the flora is not precisely known. Lactic acid bacteria and coliforms (gram-negative rods that ferment lactose to acid and gas) are the predominant micro-organisms in infant humans. Conventional (phenotypic) bacteriological analysis suggests that strict anaerobic bacteria outnumber aerobes in adults (table I). However, a considerable number of species that are observed by microscopic examination of diluted faecal specimens cannot be grown in culture media. Molecular techniques and 16S rDNA sequence analysis (genotype) indicate clustering within three main bacterial groups : Bacteroides, Gram positives related to *Clostridium coccoides* and Gram positives related to *Clostridium leptum*.(16) Mucosa-associated bacteria may differ from the community recovered from feces.(17)

The luminal environment, including the indigenous flora is separated from the internal milieu of the host by the mucosal barrier. Physically, the barrier is composed of epithelial cells (with rapid turnover) maintained together through tight and adherens junctions, a mucus layer (with trefoil peptides, mucin glycoproteins, surfactant lipids, immunoglobulin A ...) covering the surface, the vascular endothelium, an important system of innate defence including Paneth cells producing lysozyme and antimicrobial molecules such as defensins and the organized gut associated lymphoid tissue (GALT). The interaction between host and bacteria infers important health benefits to the human host and the gut flora has important and specific functions in host homeostasis. These include metabolic functions (fermentation of nondigestible dietary residue, production of vitamin K, absorption of calcium, iron etc.), protective functions (against pathogens), the development of gastrointestinal motility and trophic functions (control of epithelial cell proliferation and differentiation, development and homeostasis of the immune system). The presence of GALT and of immune competent cells in the lamina propria of the mucosa is the expression of a “controlled - physiological inflammation” in the normal gut. It maintains the gut structure and function. Bacterial colonization of the gut with diverse intestinal microbes is necessary for the development of this gut defence system. It induces the synthesis and secretion of polymeric immunoglobulin A and the generation of a balanced T helper (Th) cell response to protect the intestinal surface and inhibition of the systemic response to commensal bacteria and food proteins (oral tolerance) to prevent chronic inflammation.(18) Dysregulation of the mucosal immune response can switch a controlled inflammation into an “uncontrolled” inflammation.(19)

The indigenous or commensal flora is expected to remain in the lumen or at most, be associated with the mucus layer. The host organism is able to distinguish the indigenous flora associated with the mucus layer from pathogens interacting with the epithelial surface through the innate immune system. This system is equipped with “pattern-recognition receptors (PRRs) capable of detecting microbial motifs, called “pathogen-associated molecular patterns (PAMPs). The Toll-like receptors (TLRs) and the protein corresponding to CARD15 are well known PRRs. TLR2 (peptidoglycan, lipoprotein, zymosan and lipoarabinomannan) and TLR4 (lipopolysaccharide, taxol, lipoteichoic acid) have been shown to be essential for the recognition of distinct bacterial cell wall components. CARD15 has binding activity for bacterial muramylpeptide. Toll-like receptors are extracellular receptors while the CARD15 protein is an intracellular receptor present in monocytes, dendritic cells, epithelial cells and

Paneth cells. The CARD15 protein activates the transcription factor NF- κ B. Surface epithelial cells of the intestine either lack or have down-regulated expression of TLRs. Instead, these receptors are present within epithelial cells of the normally sterile crypts.

Infection and inflammation

Pathogens interact more intimately with the epithelial surface and/or they can actively penetrate the barrier and/or induce tissue necrosis (danger theory).

Interaction with the epithelial cells triggers a cascade of signalling events within the host cell. This may lead to the formation of an actin-rich pedestal upon which the pathogen resides. Pedestal formation requires cytoskeletal protein recruitment (CD44, tropomyosin...).⁽²⁰⁾ Human tropomyosin 5 (hTM5) is the predominant colonic epithelial cell tropomyosin isoform. It is an intracellular protein. In vitro studies have shown the presence of serum and mucosal IgG and a significant T cell response against hTM5 in ulcerative colitis (cell surface expression of hTM5 has been demonstrated in freshly isolated colonic but not small intestinal epithelial cells).⁽²¹⁾

Penetration of the barrier can be the result of different strategies (binding to outer membrane proteins – Yersinia; macropinocytosis – Salmonella). After infection with several strains of enteroinvasive bacteria (Salmonella, enteroinvasive E coli, Yersinia, Shigella, Listeria) intestinal epithelial cells rapidly (within 2 – 3 h) up-regulate the expression of a program of host genes, the products of which activate mucosal immune and inflammatory responses. This program includes the up-regulation and production of pro-inflammatory and chemo-attractant cytokines (TNF α , IL-8, growth-related oncogene α (GRO α), monocyte chemo-attractant protein-1 (MCP-1), cyclooxygenase (COX-2), prostaglandins E2 and F2 α , nitric oxide synthase (NOS) and increased surface expression of the adhesion molecule ICAM-1. This up-regulated expression is mediated by a common signal transduction pathway. NF- κ B is the central regulator of this response. Bacterial invasion is further associated with the release of antigens that signal to the underlying immune cells.⁽²²⁾

Tissue necrosis is associated with the release of shock proteins, cytokines and intracellular components, which activate antigen-presenting cells (APC), which are responsible for initiating the inflammatory reaction. This is the basis of a theory for the pathogenesis of Crohn's disease whereby it is proposed that bacterial antigens cross the epithelium as a primary or secondary phenomenon. The bacterial antigens induce lamina propria antigen presenting cells to release interleukin 12 which maintains the Th-1 cell response in the lamina propria and also present bacterial antigens to T cells. T cells accumulate and secrete interferon- γ and tumour necrosis factor- α . Increased local concentrations of these cytokines then begin the biochemical cascade which eventually produces tissue injury.⁽²³⁾

Some of the bacteria of the gut flora are potential pathogens. They can be a source of infection when the integrity of the mucosal barrier is compromised (or when the composition of the microflora is changed?).

The bowel flora in IBD

The faecal flora of IBD patients was the topic of studies already many years ago. These studies used classical bacteriological techniques aimed at the identification of the bacteria through their phenotype. One of the earliest micro-organisms incriminated in ulcerative colitis was the "diplostreptococcus". This organism was found in the faeces of colitis patients and intravenous challenge in rabbits produced ulcerative disease of the large bowel. This was an early attempt to prove the transmissible nature of a potential causative agent.⁽²⁴⁾ Among the normal flora components, coliforms were shown to be increased in the faecal effluent of patients with UC, especially during periods of relapse. In general, UC

patients tended to have one serotype of coliforms with adhesive or invasive properties (normal controls had several serotypes) that dominated the faecal flora.(25)

If bacteria are involved in the pathogenesis of IBD, the bacteria or bacterial components are however more likely present in the tissue or closely associated to the mucosa. A first attempt to identify bacteria associated with the tissue was the culture of biopsies obtained by endoscopic biopsy or surgery. In samples of ileal serosa and mesenteric lymph nodes harvested at surgery, bacteria were found in serosal tissues of 27% of CD patients (15% in controls) and in the mesenteric lymph nodes of 33% CD patients (5% controls). The types of organisms generally were those expected in the normal flora, such as *E. coli*, *Proteus*, *Bacteroides* and *Streptococci*.(26, 27) The findings were confirmed in another study using mesenteric lymph nodes. Bacteria were present in 48% of the lymph nodes. *E. coli* was the most common organism (27%). Bacterial translocation due to surgical manipulation may however explain the findings too (28)

Several authors looked at circulating antibodies and found high titres against normal flora bacteria in IBD patients. Among the aerobic bacteria, antibodies to specific serotypes of *E. coli* and *Streptococcus faecalis* were found to be elevated.(29)

Using real-time quantitative polymerase chain reaction (PCR) and formalin-fixed paraffin-embedded tissue samples, *Bacteroides vulgatus* and *E. coli* were detected more frequently and in greater numbers in samples from patients with IBD. Frequency and numbers were not related to the severity of the disease. *E. coli* was found in perivascular areas, in the muscle layer and in germinal centers of lymph follicles.(30) Using homogenized tissue from biopsies, culture and additional techniques, colonization of intestinal lesions (ulcers and fissures) by *E. coli* and streptococci was demonstrated in over 60% of the cases studied. *E. coli* strains are able to adhere to and invade cultured intestinal epithelial cells. Furthermore they are able to survive and replicate within macrophages without inducing host cell death. Adherent *E. coli* strains may disrupt the intestinal barrier by synthesizing an alpha-haemolysin.(31, 32)

With laser capture micro-dissection and PCR, *E. coli* DNA (analysis of the genotype of bacteria and not the phenotype) was detected more frequently in Crohn's granulomas (80% - 12/15) than in other non-Crohn's bowel granulomas (1/10).(33) Using a similar micro-dissection technique and PCR amplifying the full length of 16S rDNA with universal primers, we detected bacterial DNA in 36 samples from all 5 patients with CD examined (including normal mucosa; mucosa-associated and submucosal lymphoid aggregates, ulcers, granulomas and mesenteric lymph nodes). Subsequent cloning and sequence analysis revealed the presence of aerobic and anaerobic species and a larger diversity in the colon vs. the small bowel. The most common isolates were *Bacteroides* and *Clostridium* species and *E. Coli*. *Pseudomonas fluorescens* was found in an aphthoid ulcer.

An indirect argument for the implication of bacteria is the finding that the natural antimicrobial defensin DEFA5 and DEFA6 genes are particularly over-expressed in CD. The number of patients studied for global gene expression profiles is however low and patients with genetic defects may not have been present in the group examined.(34)

In summary, bacteria have repeatedly been recovered from samples from patients with IBD. Different bacteria have been recovered from the tissue using a variety of techniques. While *E. coli* has been recovered repeatedly and frequently and thus may be a good candidate, it is also a common bacterium and there are different strains with varying virulence factors. Furthermore, some data are not in favour of *E. Coli*. Using a technique called serological position cloning which screen DNA libraries with defined antisera, strong reactivity against specific flagellins (recognized by TLR5) was observed in animal models. The two flagellins tested were Cbir1 and FLA-X possibly derived from bacteria in the *Clostridium* and related genera (which are common in the bowel) but different to *Salmonella* species or *E. coli*

flagellin. Remarkably, serum IgG to Cbir1 flagellin was also found elevated in patients with CD (50%) but not in UC or infective colitis and normal controls.(35) These need however confirmation.

The bowel flora and inflammation in IBD – different possible pathways

Impaired recognition of bacterial components by a compromised innate immune system may lead to histologically recognizable lesions. CARD15(NOD2) mutations have been identified in a subgroup of patients presenting with Crohn's disease. The mutations are associated with diminished mucosal α -defensin expression. Human α -defensins (HD-5 and HD-6) are antibiotic effector molecules normally predominantly expressed in Paneth cells of the ileum. A decreased expression might be responsible for a break in mucosal tolerance towards various luminal bacteria.(36) The dominant over-expression of the anti-microbial defensins in Crohn's disease observed in gene expression profiles is not necessarily in contradiction with this finding. It is thus far reported mainly for samples from the colon. An enhanced expression in the colon has been noted in the colon. Pro-inflammatory cytokines can enhance this expression.(37) Defensins are T cell chemotactic and may help sustain recruitment of T cells in the mucosa.

No association was found between CARD15 and TLR4 variants and the presence of granulomas in CD.(38) The CARD15 protein activates the transcription factor NF- κ B which plays a pivotal role in the inflammatory reaction. Aberrant NF- κ B signalling may be relevant for the development of CD but this theory needs confirmation.(39) Signalling via mutated CARD15 proteins leads to defective activation of NF- κ B and defective T helper 1 (Th1) response which facilitates infection. However, CD is characterized by increased Th1 response. This may be due to increased activation of NF- κ B through increased TLR2 activation of NF- κ B, a process which is normally inhibited by intact CARD15 signaling.(40)

Crohn's disease is characterized by hyper-activation of Th1-cells with abundant secretion of interferon- γ and tumour necrosis factor- α and B-cells producing IgG2a and IgG2b antibodies directed against commensal bacteria. The induction of an efficient T-cell response requires the interaction with professional antigen-presenting cells, such as dendritic cells (DC). They are key regulators of the balance between tolerance and immunity. Immature or resting DC may present antigens in a tolerogenic fashion whereas mature (activated) DC are capable to induce T-cell immunity. A DC population characterized by high expression of the tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) has been shown to exert potent inhibitory effects on T-cell survival and proliferation in vitro and in vivo. IDO+ DC are normally present in tissues such as non-inflamed tonsils. In lesional colonic biopsies of Crohn's disease patients, IDO mRNA is increased (more than in ulcerative colitis). A reduced IDO expression is observed in samples from patients with Crohn's disease with good clinical response to infliximab. IDO induction might contribute to a negative feed-back loop by inhibition of further T-cell activation and thus promote persistent inflammation.(41) Further evidence for an imbalance in intestinal DC subpopulations has been observed in immuno-histochemical studies demonstrating a reduction in CD11c+ and CD83+ (mature) DC in CD tissue samples compared with controls.(42)

Recent evidence indicates that defective T-cell apoptosis may be an important factor in CD.

TNF α (secreted by T cells) has an important role in regulating intestinal barrier function (transcellular and paracellular).(43) It may mediate an increased epithelial uptake of protein antigen in the ileum of CD. Such an event could accelerate the process in the early stage of the mucosal inflammation. The reduced protection against luminal bacteria (reduced defensin expression) and a hyperreactive immune system giving an elevated background of

TNF α with epithelial barrier dysfunction as a consequence would promote persistent inflammation.(44)

Evidence for a defect in the immune system can further be found in the nature of the early lesions observed in Crohn's disease. The so called aphthoid ulcers often involve the epithelium overlying mucosal lymphoid aggregates. These areas contain M-cells which are important for the development of an immune response towards viruses and bacteria. Antibodies to the Crohn's disease-related bacterial sequence (I2) and anti-Escherichia coli outer membrane porin Care associated with CD phenotypes, and patients with the highest level of serum reactivity towards an increasing number of microbiota have the greatest frequency of strictures, internal perforation and small bowel surgery.(45) In addition, repeated damage and injury of the intestinal surface, a characteristic feature of IBD, may facilitate the entry of luminal antigens and the perpetuation of the inflammatory response.

A better understanding of and enhancement of intestinal repair mechanisms may therefore also provide a new approach for the treatment of IBD.

Conclusion

Bacteria are frequently recovered in patients with IBD. Thusfar no single species could be implicated in the pathogenesis. Intolerance towards the indigenous flora may be more likely. This may induce inflammation through different mechanisms.

Table I : Predominant anaerobes found in the colon

Bacteroides	Eubacteria
Bifidobacteria	Clostridia
Lactobacilli	Ruminococci
Peptostreptococci	Peptococci

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