The Role of Infection in Inflammatory Bowel Disease: Initiation, Exacerbation and Protection

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ABSTRACT: Inflammatory bowel disease, a collective term for ulcerative colitis and Crohn's disease, is a chronic, immune-mediated disease of the gastrointestinal tract that develops in genetically susceptible individuals. The role of infection in the development of inflammatory bowel disease is underscored by various clinical observations, such as the delayed age of onset, suggesting that childhood exposure to pathogens is essential, and the clinical improvement that follows decreasing bacterial intestinal load. Over the years, many a pathogen has been linked to the development and exacerbation of inflammatory bowel disease, notably; *Mycobacterium paratuberculosis, Escherichia coli, Listeria monocytogenes* and Chlamydia as well as viruses such as measles, mumps, rubella, Epstein-Barr virus and cytomegalovirus. Presently, leading theories of disease pathogenesis suggest loss of immune tolerance to normal commensal bacteria coupled with excessive exposure to bacterial antigenic products. This review describes the most commonly implicated pathogens in the causation of IBD and presents the evidence supporting their pathogenic role as well as data that offset their importance.

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The association between infection and autoimmunity, the first leading to the latter by the mechanism of molecular mimicry, epitope spreading, and bystander or polyclonal activation, is well established. The role of infection may be primary, as exemplified by streptococcal infection and rheumatic fever, the infection being a prerequisite for disease initiation in a genetically susceptible host, or secondary, where the causation is vague and is suggested by an excess of positive serology for a certain pathogen as in the implication of diabetes mellitus and Epstein-Barr virus infection. A myriad of pathogens have been implicated in the causation and exacerbation of inflammatory bowel disease; microbial agents play a role in most of the prevalent etiologic hypotheses and animal disease models of both Crohn's disease and ulcerative colitis. Over the years, many organisms have been proposed as etiologic agents for IBD, to the extent of suggesting that CD, for example, is a manifestation of chronic mycobacterial infection [1]. Yet, although persistent infection with a specific pathogen cannot be excluded, most agents proposed to date were based on observational studies and were not confirmed by subsequent laboratory investigations. In the last decade, the paradigm has shifted from infectious agents to loss of immune tolerance to commensal enteric bacteria. This hypothesis is strengthened by the observation that the composition of the enteric flora is altered in IBD with increased number of aggressive bacteria such as Bacteroides, adherent/invasive *Escherichia coli* and enterococci, and decreased number of protective lactobacilli and bifidobacteria [2]. Additional observations that highlight the role of commensal bacteria in the etiopathogenesis of IBD include the beneficial effects of antibiotics on the course of CD as well as the symptomatic improvement that follows diversion of the fecal stream and recurrence of inflammation that follows restoration of intestinal continuity [3]. The contribution of host genetic susceptibility gains momentum by the recent association of CD with mutations in the *NOD2* gene, whose products are bacteria-recognizing proteins [4]. Conversely, infection with *H. pylori* and helminthic infections, the latter by shifting the immune response to the Th2 phenotype, ameliorates clinical and experimental IBD, respectively. In this review we describe the role of infectious agents and commensal bacteria in the initiation and exacerbation of IBD on the one hand, and in protection from disease on the other.

IBD = inflammatory bowel disease

CD = Crohn's disease

THE ROLE OF INFECTION IN ANIMAL MODELS

The concept that normal enteric bacteria initiate and sustain inflammation in a genetically susceptible host is based on a landmark study in rodents showing that HLA-B27 transgenic rats spontaneously develop chronic colitis, whereas the same rats in a germ-free environment are protected from disease [5]. Conversely, pretreatment with antibiotics has been shown

to alleviate intestinal inflammation in animal models [6]. Particularly, the presence of some anaerobic species appears to be critical for the development of transmural inflammatory lesions in this model, as these lesions are not observed in the absence of anaerobic bacteria strains. The importance of the mucosal barrier is underscored by the genetic susceptibility of rodents harboring a genetic defect in mucosal barrier function [7]. Taken together, these models highlight the roles of an intact mucosal barrier and commensal anaerobes in transmural inflammation. Probiotics are living microorganisms that upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition. In contrast to anaerobic bacteria, probiotics such as *L. reuteri* and *L. salivarius* were associated with reduction of mucosal inflammatory activity in animal models of bowel inflammation [8].

Numerous infectious agents, including viruses, bacteria and parasites, have been linked to the development and exacerbation of inflammatory bowel disease

BACTERIA IMPLICATED IN THE CAUSATION OR EXACERBATION OF IBD

MYCOBACTERIUM PARATUBERCULOSIS

The histopathological similarities between CD and mycobacterial infection, notably the epithelial granulomata as well as the macroscopic lesions of segmental and fibrosing stenosis, have led investigators to implicate the latter in the pathogenesis of IBD. This compelling hypothesis has prompted a large number of epidemiological studies searching for *Mycobacterium avium* subsp. *paratuberculosis* in patient tissue, which, in compilation, have neither confirmed nor dismissed an association between the two diseases [9,10]. However, the issue has not been laid to rest and studies of the association between IBD and MAP continue to emerge. Recently, antibodies against a recombinant protein encoding an insertion element specific for MAP were found to be significantly higher in Japanese patients with CD than in those with ulcerative colitis, colonic tuberculosis, and control subjects [11]. In addition, it was postulated that the efficacy of both methotrexate and 6-mercaptopurine in inhibiting MAP growth *in vitro* explains the physiology behind their beneficial clinical effect in patients with IBD [12]. Finally, the discovery of a number of Crohn's susceptibility genes, including *NOD2/CARD15*, demonstrates that Crohn's is a complex genetic disease. As mutations in *NOD2/CARD15* do not necessarily lead to Crohn's disease, other mitigating factors, genetic and/or environmental, are probably required to produce illness. Recent work has shown that *NOD2/CARD15* plays a role in bacterial sensing and activation of innate immune responses, providing a link between Crohn's genetics and an environmental factor, potentially a bacterial trigger [13]. Similarly, the presence of MAP and of the natural resistance-associated macrophage protein 1 (NRAMP1), now SCL11A1, and gene polymorphisms, were found to be strongly associated with CD, highlighting the interplay of infectious and genetic factors in disease pathogenesis [14].

This exogenic microbial exposure coupled with a genetic susceptibility that leads to loss of immune tolerance to normal commensal bacteria is considered the basis for the development of IBD

E. COLI

E. coli, the predominant aerobic gram-negative species of the normal intestinal flora, abnormally colonizes the terminal ileum in CD patients and is abundant in both early and chronic ileal lesions in these patients. In addition, antibody titers against *E. coli* are higher in CD patients than in controls, especially against the *E. coli* outer membrane protein C, and are associated with more severe disease [15]. The invasive nature of *E. coli* strains isolated from ileal lesions (designated adherent-invasive *E. coli*) supports their putative role in the initiation of CD. Moreover, AIEC strains isolated from these patients are able to survive and replicate extensively within murine macrophages, whereas most invasive bacteria (such as Shigella, Salmonella and Yersinia) induce cell death of infected macrophages [16]. The high replication of AIEC bacteria within macrophages leads to excessive production of tumor necrosis factor-alpha granuloma formation [16]. Indeed, *E. coli* antigens and DNA were detected in 80% of CD granulomas [17]. In conclusion, the high prevalence of AIEC in patients with ileal involvement of CD, their ability for intracellular replication, induction of TNFα and granuloma formation links them to the pathogenicity of CD perhaps against the background of a dysfunctional *NOD2* innate immune surveillance mechanism.

LISTERIA MONOCYTOGENES

L. monocytogenes has been suggested as an organism with the potential to cause and exacerbate IBD after it was found at the site of perforation in the colon of a patient with fulminant ulcerative colitis [18]. However, a later study found equal prevalence of *L. monocytogenes* DNA in the intestine of patients with IBD and in non-IBD control patients, presumably reflecting the widespread presence of this organism in the environment [19]. This, in conjunction

MAP = *Mycobacterium avium* subsp. *paratuberculosis*

AIEC = adherent-invasive *E. coli*
TNFα = tumor necrosis factor-alpha

with the low yield of positive biopsies for *L. monocytogenes* in IBD does not support a direct role for this pathogen in the pathogenesis of IBD [19]

CHLAMYDIA

Chlamydia is another organism of autoimmune potential thought to participate in the pathogenicity of IBD. However, no marked differences in titers of anti-*C. pneumoniae* serum immunoglobulin G were found between healthy controls and patients with IBD [20]. As for *C. pneumoniae* DNA, it was detected in 9 of 42 biopsies from patients with CD (21.4%), 9 of 59 biopsies from patients with UC (15.3%), and 14 of 122 biopsies from non-IBD control patients (11.4%) [21], while others have found equal frequencies of the pathogen [20]. Interestingly, in CD patients, *C. pneumoniae* DNA was more prevalent in inflamed tissue specimens compared with unaffected areas [20]. Taken together, the interpretation of these findings is that although *C. pneumoniae* is unlikely to be of pathogenic importance in IBD, it may still influence local clinical manifestations.

Increased antibiotic use and improved hygiene, which alter the balance of beneficial versus aggressive microbial species, contributes to the increasing prevalence of IBD in the western hemisphere

BLASTOCYSTIS HOMINIS

Recently, a pathogenic variant of Blastocystis, a common intestinal organism, was described in patients experiencing IBD-like, chronic gastrointestinal symptoms [22]. Existing clinical methods often fail to detect infection with this organism, leading to misdiagnosis of cases as "idiopathic" IBD. It is hypothesized that a virulent strain of this organism emerged in the Middle East in the 1990s and was later transmitted to Europe and the United States by military and business travelers, giving rise to the increasing incidence of IBD in Europe at that time.

CLOSTRIDIUM DIFFICILE

C. difficile should be sought in IBD patients with active colitis. Several reports have noted an increased incidence of infection with this agent in IBD patients, 61% of whom give a history of recent antibiotic exposure. More importantly, the majority of those infected require hospitalization, and 20% require colectomy [23].

VIRUSES IMPLICATED IN THE CAUSATION OF IBD

MEASLES

Considerable attention has focused on the role of measles infection and/or vaccination in the pathogenesis of ulcerative colitis and Crohn's disease, particularly in view of the increasing incidence of the latter. The first cohort study, published in 1995 [24], found an association between measles vaccination and IBD, which was not corroborated by a later case-control study [25]. The association between measles infection and IBD is also contentious. An initial report of high rates of Crohn's disease among pregnancies affected by measles infection [26] was followed by negative studies [27]. The same discrepancy is found in laboratory investigations; while some investigators claimed to find persistent measles infection among patients with IBD, others, using highly sensitive polymerase chain reaction techniques [28], were not able to replicate the findings. In summary, available evidence does not support an association between measles-containing vaccines and risk of IBD, nor between measles infection and IBD.

MUMPS

The British Cohort Study that claimed that atypical measles infection in childhood was a risk factor for IBD also implicated mumps infection before age 2 years with an increased risk for ulcerative colitis (odds ratio 25) [29]. However, the mumps virus genome was not detected by reverse transcriptase-PCR in intestinal specimens of IBD patients, and anti-mumps IgG titer was not significantly different between patients and controls, weakening the causal link between persistent mumps virus infection and IBD [30].

Another study that assessed seropositivity for measles, mumps and rubella among IBD patients versus controls found no association between having acquired measles, mumps or rubella (by natural infection or through vaccination) and CD or UC. There was even suggestion of a protective effect of having acquired rubella infection or vaccine, against acquiring CD [31].

EPSTEIN-BARR VIRUS

Epstein-Barr virus infection is associated with infectious mononucleosis, lymphomas, gastric adenocarcinomas and a myriad of host immune responses. Interest in the potential role of EBV infection in the etiopathogenesis of IBD was sparked by the detection of increased numbers of EBV-infected B lymphocytes in intestinal mucosal samples affected by ulcerative colitis and, to a lesser extent, Crohn's disease [32].

The link between immunosuppressive therapy and lymphoma in patients with solid organ transplantation is well established and attributed to unchecked proliferation of

UC = ulcerative colitis

PCR = polymerase chain reaction
Ig = immunoglobulin
EBV = Epstein-Barr virus

Table 1. Infectious agents implicated in the pathogenesis of IBD: supporting and opposing evidence

Pathogen	Supporting evidence	Opposing evidence
Mycobacterium paratuberculosis	• Histopathology resembling CD • Higher anti-MAP antibody levels in CD patients • Clinical efficacy of MTX and 6MP in IBD coupled with *in vitro* efficacy against MAP suggesting causality • Similarity between MAP and natural resistance-associated macrophage protein 1 (NRAMP1)	• Non-contributory epidemiological studies
E. coli	• Colonizes terminal ileum in CD • High antibody titers and DNA in CD • Association with adherent-invasive *E. coli* (AIEC), which replicates within, rather than kills, macrophages – leading to typical granuloma formation	• Unknown
Listeria monocytogenes	• Organism identified at site of UC colonic perforation in a single reported case	• Equal prevalence of Listeria DNA in IBD and non IBD patients, rarely found in intestinal biopsies
Chlamydia	• Chlamydia-DNA more prevalent in inflamed as opposed to uninflamed tissue in CD patients	• No difference in antibody titers among patients and controls; equivocal findings regarding presence of DNA in intestinal biopsies
Blastocystis hominis	• A pathogenic variant described in patients experiencing "IBD-like" symptoms • Increasing incidence of IBD in Europe putatively associated with transmission of agent by military and business personnel	• Unknown
Clostridium difficile	• Increased incidence of infection with Clostridium in active IBD although not directly implicated in pathogenesis	• Unknown
Measles	• Association between measles vaccination and development of IBD in a cohort study • High rate of CD in pregnancies affected by measles infection • Evidence of persistence measles infection in IBD patients	• Association with vaccination not confirmed in a case-control study; association between infection and pregnancy not corroborated in later studies while PCR-based techniques rule out persistent infection
Mumps	• Association between childhood mumps infection and UC in adulthood, in a cohort study	• No difference in anti-mumps antibody titers or in detection of viral DNA in intestinal specimens between patients and controls
Epstein-Barr virus	• Increased EBV-infected lymphocytes in intestine of UC, and to a lesser extent CD, patients • Increased risk of lymphoma in IBD is perhaps mediated by EBV infection	• Unknown
Cytomegalovirus	• CMV infection may present as IBD disease flare-up, yet antiviral therapy and not immunosuppressants is required to attain a cure	• Unknown
Saccharomyces cerevisiae	• Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are associated with CD	• Antibody response may reflect an epiphenomenon resulting from an immunological response against an unrecognized antigen
Helminthes	• Putative protective factor as prevalence of IBD is lower in underdeveloped countries in which intestinal infestation with nematodes is common • Nematode infection ameliorates experimental murine colitis	• Unknown
Helicobacter pylori	• Decreased prevalence of seropositivity in IBD patients suggests a protective modifier effect for *H. pylori* infection	• Unknown

EBV-infected lymphocytes. Concerns regarding the increased risk of lymphoma among IBD patients are well founded, especially in individuals treated with multiple immunomodulators and biological agents, underscoring the role of surveillance.

CYTOMEGALOVIRUS

Cytomegalovirus infection may present as a flare-up of IBD not responding to traditional therapy, thus leading to a high rate of colectomy (67%) and mortality (33%) [33]. The prevalence of infection in acute severe colitis ranges between 21% and 34%, with remission rates of 67–100% following antiviral therapy with intravenous ganciclovir [34]. It is imperative that concurrent cytomegalovirus infection be considered in IBD patients with acute colitis, especially in a medically immunosuppressed host.

YEAST IMPLICATED IN THE CAUSATION OF IBD

Saccharomyces cerevisiae, used in baker's yeast, was considered an innocuous component of our food until the presence of anti-*Saccharomyces cerevisiae* antibodies were first described in patients with CD [35]. While ASCA are thought to result from a specific antibody response to the *S. cerevisiae* cell wall mannan, it is still unclear whether this is a direct response to the yeast itself or an epiphenomenon resulting from a similar immunologic response to another antigen. For example, gastrointestinal colonization with *Candida albicans*, which promotes sensitization against food antigens in mice, was recently shown to be one of several immunogens for ASCA [36] and may be at the origin of the aberrant immune response in CD.

ASCA = anti-*Saccharomyces cerevisiae* antibodies

INFECTIOUS AGENTS CONFERRING PROTECTION AGAINST IBD

PARASITES

IBD is common in temperate regions and rare in tropical countries affected by poor sanitation and overcrowding; immigrants typically acquire the higher risk of IBD associated with the adopted, developed country [37]. These observations overlap the prevalence of chronic enteric infestation with parasites, which is high in underdeveloped countries and rare in industrialized countries due to improved hygienic conditions. The contrasting geographic distributions of nematode infection and IBD suggest that generation of a Th2 response by nematodes ameliorates Th1-mediated diseases, such as CD, and lowers their occurrence. This hypothesis was strengthened by an elegant study on experimental murine colitis, which showed that nematode infection indeed reduced the severity of colitis and decreased the resulting mortality [38].

HELICOBACTER PYLORI

Several reports have documented a decreased prevalence of *H. pylori* seropositivity in IBD patients [39], decreased even further by sulphasalazine treatment. On a similar note, some suggest that the higher age of onset and bimodal pattern of age-specific incidence in seropositive IBD patients are the result of a protective modifier effect that *H. pylori* infection exerts on the development of IBD [40].

The supporting and opposing evidence for the role of the various infectious agents in the pathogenesis of IBD is presented in Table 1.

SUMMARY

The role of infection in inflammatory bowel disease is complex. On the one hand, infection with a pathogenic organism could serve as an environmental trigger to initiate an inflammatory response, a response that may be perpetuated in a susceptible host by commensal microbial antigens. On the other hand, host genetic susceptibility, in the form of a defective mucosal barrier function, can lead to enhanced exposure to luminal bacteria. Either process can lead to an overly aggressive T cell response to normal bacteria, culminating in tissue damage. It seems, therefore, that it is the combined effect of genetic susceptibility with microbial exposure, in addition to increased antibiotic use and improved hygiene, that alters the balance of beneficial versus aggressive microbial species, that perpetuates the pathophysiology of IBD and contributes to its increasing prevalence in the Western hemisphere. Thus, although many pathogens have been implicated in the causation of IBD, not a single pathogen, in itself, is responsible for the disease.

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