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REVIEW

Pathogenesis of Crohn's disease: Bug or no bug

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Abstract

The possibility of an infectious origin in inflammatory bowel disease (IBD) has been postulated since the first description of Crohn's disease (CD). Many observations implicate bacteria as a trigger for the development of CD: lesions occur in regions with higher bacterial concentrations; aphthous ulcers occur in Peyer's patches; inflammation resolves when the fecal stream is diverted and is reactivated following reinfusion of bowel contents; severity of the disease is correlated with bacterial density in the mucosa; granulomas can contain bacteria; and susceptible mice raised in germ-free conditions develop inflammation when bacteria are introduced in the 1990's, several studies sought to establish a relationship with viral infections and the onset of IBD, finally concluding that no direct link had been demonstrated. In the past fifteen years, evidence relating IBD pathogenesis to Mycobacterium avium paratuberculosis, salmonella, campylobacter, *etc.*, has been found. The tendency now under discussion to regard microbiota as the primary catalyst has led to the latest studies on microbiota as pathogens, focusing on *Escherichia coli*, mainly in ileal CD. The present review discusses the literature available on these "bugs".

Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Bacteria; Virus; Pathogenesis

Core tip: The possibility of an infectious origin in inflammatory bowel disease (IBD) has been postulated since the first description of Crohn's disease (CD). Many observations implicate bacteria as a trigger for the development of CD, and have tried to do so with virus. Inconclusive evidence relating IBD pathogenesis to Mycobacterium avium paratuberculosis, salmonella, campylobacter, *etc.*, has been found. The tendency now under discussion to regard microbiota as the primary catalyst, has led to the latest studies on microbiota as pathogens, focusing on *Escherichia coli*, mainly in ileal CD. The present review discusses the literature available on these "bugs".

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INTRODUCTION

Inflammatory bowel diseases (IBD), mainly Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders of the gastrointestinal tract[1]. Extensive studies in the past decades have suggested that the etiology of IBD involves environmental and genetic factors that lead to dysfunction of the epithelial barrier with consequent deregulation of the mucosal immune system and responses to gut microbiota[2].

Genome-wide association studies have shown that many genes correlate with the development of CD and UC, although not every individual presenting genetic abnormalities will develop the disease. Other factors, such as environmental triggers must play a role[3]. Dietary alterations in the Western populations have resulted in a shift in the composite gut microbiota. Colonic bacteria have a metabolic function, with a symbiotic relationship with human beings[4]. Gut microbiota, which outnumber human cells by nearly ten-fold and contain more than one million genes[4,5], have been shown to play an important role in complex disorders such as IBD. Historical animal models of CD initiated granulomatous change in both mice and rabbits by infiltrating healthy animal tissue with human Crohn's tissue[6-8]. Work with animals has demonstrated the ability of an organism to induce colitis in immunodeficient but not immunocompetent mice[9]. This highlights the importance of genetics, and underlines the need to understand the hosts' conditions, and might explain why studies have previously been unable to find a specific pathogen[4].

As mentioned, IBD is thought to be the result of a combination of genetic predisposition interacting with environmental factors that modify the gut microbiota[3]. One of the explanations for the onset of IBD suggests a three-step scenario, in which bacteria penetrate the epithelial barrier, provoking a weak inflammatory response with impaired clearance, which in turn causes chronic inflammation, culminating in IBD[10].

It has become evident that IBD patients show an intestinal dysbiosis, with a decrease in the number of potentially beneficial bacteria such as *Bifidobacteria, Lactobacilli* and *Firmicutes,* and an increase in that of putative pathogenic bacteria such as *Escherichia coli* (*E. coli*) and other Enterobacteria[11,12]. Whether gut microbiota are responsible for the onset of IBD or a mere consequence of pathogenic microbial enteric-wall invasion, remains to be elucidated.

Over the years, many organisms have been proposed as etiological agents for IBD, to the extent of suggesting that CD, for example, is a manifestation of chronic mycobacterial infection[13-18] or *E. coli* adherence and invasion[19-23]. Many observations implicate bacteria as a trigger for the development of CD: lesions occur in regions with the highest bacterial concentrations; aphthous ulcers, the earliest lesion in CD, occur in Peyer's patches, the site of bacterial sampling; inflammation resolves when the faecal stream is diverted and is reactivated following reinfusion of bowel contents[24]; severity of the disease is correlated with bacterial density in the mucosa; granulomas contain bacteria; and susceptible mice raised in germ-free conditions only develop inflammation when non-pathogenic bacteria are introduced[24-26].

The possibility of an infectious origin in IBD has been postulated since Dalziel's first description of CD in 1913. He compared CD with Johne's disease in cattle, caused by *Mycobacterium Avium Paratuberculosis*[4]. Many studies have tried to find a germ that is responsible for IBD. The most recent papers focus on *E. coli*, especially in ileal CD, but there is literature for and against several bacteria (*mycobacterias*, *helicobacters*, *campylobacter*, *etc.*) and viruses (Ebstein-Barr virus, Cytomegalovirus, paramyxoviruses, *etc.*), as causative agents (Table 1). In this article, we will summarize the conclusions regarding these agents found in the medical literature.

ROLE OF BACTERIA IN THE ETIOLOGY OF IBD

Adherent-invasive E. coli

E. coli is the predominant aerobic Gram negative species of the normal intestinal flora, where it plays an important role in promoting the stability of the intestinal microbial flora and in maintaining the normal intestinal physiology. By acquisition of virulence factors, such as production of enterotoxins and cytotoxins, tissue invasion, and adherence to enterocytes, *E. coli* strains become pathogenic and become involved in intestinal diseases[27].

Interest in *Escherichia coli* as a pathogen in IBD began with the observation that organisms isolated from patients with CD had greater adherent properties to human cells than those from controls, and that previously unrecognized invasive *E. coli* were present in Crohn's ileal tissue[4,19,25].

Higher *E. coli* antibody titres and *E. coli* antigens have been found in the blood and resection specimens, respectively, of CD patients[28,29]. In Darfeuille-Michaud's study, *E. coli* was recovered from 65% of chronic lesions in resected ileum, and 100% of biopsies of early lesions, in postoperative endoscopic recurrence.

As mentioned, *E. coli* form a major component of the normal microflora of the gut. However, the *E. coli* prevalent in CD tissue was shown to have unique adherent and invasive properties, which enabled it to adhere to intestinal epithelial cells (IECs), invade the epithelial layer, and replicate within both IEC and macrophages[30]. These properties were used to designate it as a specific type of *E. coli*, adherent-

Table 1 Microorganisms involved in inflammatory bowel disease

Microorganism	Main features
Bacteria	
Adherent-invasive *Escherichia coli*	The AIEC has adherente and invasive properties. They are found in macrophages of ileal CD tissue. It adheres with a type 1 pili to CEACAM6 receptors (increased in ileal tissue). Invasion is facilitated by endomasplic reticulum expresion of Gp96 AIEC promotes translocation of bacteria and stimulates TNF production, promoting granuloma formation. CD mutations enhance intracellular replication. AIEC is very similar to the urologic UPEC
Mycobacterium avium paratuberculosis	MAP causes CD-like disease in animals. It has been found un blood and intestinal tissue of CD patients, mainly in spheroplast form (cell-wall deficient form which may take up to 18 mo to culture in special stains). Blood MAP DNA has been found in higher levels in controls, as a sign of exposure in the general population and maybe due to the treatment IBD patients receive, which has been shown to inhibit MAP. MAP antibodies have been found in IBD sera. MAP is a source for ASCA. *In vitro*, MAP impair macrophages to kill *E. coli*. Several CD genetic alterations can favor MAP infection
H. pylori	Epidemiological studies have observed an inverse correlation between IBD and HP, that cannot only be explained by coincidence or by previous antibiotic treatment. It has been shown that HP's DNA has the capacity to reduce type I IFN
C. difficile	Up to 10% of the IBD patients will develop *C. difficile* infections, 40% of them without having had previous antibiotic treatment. It is considered a risk factor for exacerbations and should be screened in every IBD patient hospitalized for a flare
Campylobacter and *salmonella*	The risk of IBD after a Campylobacter or Salmonella positive test is high, but it is also high if a stool test has been done and it was negative, suggesting that IBD patients undergo stool tests in the years before diagnosis
Virus	
Measles and mumps	Implication of these virus and their vaccines in the pathogenesis is uncertain, specially with respect to measles
Rubella	No relationship has been found
Cytomegalovirus	It reactivates underlying inflammatory disease. The intensity in which CMV is expressed in the intestinal mucosa relates to the severity of the inflammation
Epstein-Barr virus	Like CMV, it has a modulating function, not an ethiological implication

AIEC: Adherent-invasive *Escherichia coli*; MAP: *Mycobacterium avium paratuberculosis*; IBD: Inflammatory bowel disease; CD: Crohn's disease; ASCA: Antibodies against saccharomyces cerevisiae; CMV: Cytomegalovirus.

invasive *E. coli* [*Adherent-invasive escherichia coli* (AIEC)].

Studies on the adherence properties of *E. coli* have concluded that *E. coli* strains are able to adhere to various human cells or cell lines. 53%-62% of *E. coli* strains isolated from feces of CD were able to adhere to buccal cells, compared to only 5%-6% of those isolated from control subjects. A correlation between bacterial adhesion to intestinal cells and intestinal colonization has been observed. The presence of high levels of bacteria creates a biofilm on the surface of the gut mucosa in patients with CD and UC[31,32].

Analysis of *E. coli* strains isolated from early and chronic ileal lesions of patients with CD has revealed the presence of true invasive pathogens. Electron-microscopy of epithelial cells infected with CD-associated bacteria has revealed a macropinocytosis-like process of entry. Inside the host cells, CD-associated bacteria survive and replicate in the cytoplasm after lysis of the endocytic vacuole[26,31]. Glasser *et al*[21] demonstrated that AIEC was able to survive and replicate in macrophages, without inducing host cells and stimulating the infected cells to release high levels of tumor necrosis factor (TNF)-α.

Genetically related *E. coli* strains were shown to adhere and invade the intestinal wall in susceptible hosts[22]. These strains expressed type 1 pili, which presented point mutations, with amino acid substitutions in the type 1 pili FimH adhesion subunit, which contributed to the AIEC adhesion to the Carcinoembryonic antigen-related cell adhesion molecule 6 receptors (CEACAM6 receptor) in the IEC. In contrast to non-AIEC isolates, AIEC isolates tended to carry FimH hotspot mutations that were of recent evolutionary origin and could be signatures of pathoadaptive mutations[30,33].

B2 and D *E. coli* strains are more frequently pathogenic, causing urinary tract and other extra-intestinal infections. AIEC isolated from CD patients were generally found to belong to the B2 and D phylotypes. This suggested that these isolates take advantage of a specific micro-environment found in the IBD gut[33-35].

AIEC genome sequencing revealed its similarity to uropathogenic *E. coli* (UPEC)[36]. Phylogenetic analysis of fimH sequences delineated a tight S70/N78 clade containing LF82, the reference strain for AIEC. Interestingly, UPEC and avian pathogenic *E. coli* were also found in the S70/N78 clade. As Dreux *et al*[33] remarked in their paper, this presence raises the possibility that IBD-isolated *E. coli* are members of a general pool of extraintestinal pathogenic *E. coli* that reside in the gut and have evolved specific potentialities dependent upon their microenvironment.

The Darfeuille-Michaud group highlighted potential pathological mechanisms for AIEC in CD: AIEC express type 1 pili which enables binding to CEACAM6 receptors that are increased in the ileal mucosa of CD patients; in ileal intestinal epithelial cells from CD patients, strong expression of the endoplasmic reticulum stress protein Gp96 facilitates invasion *via* recognition of the bacterial outer membrane protein OmpA of AIEC[37]; AIEC carriage of the long polar fimbriae (lpf) virulence

gene promotes translocation of the bacteria across Peyer's patches[38]; and CD-associated mutations in autophagy genes enhance intracellular replication of AIEC[39]. AIEC have also been shown to induce the release of TNFα, a key cytokine in IBD inflammation. They survive and replicate inside macrophages inducing the release of large amounts of TNFα and motivating granuloma formation *in vitro*[21,40]. Recently, infection with AIEC was proven to up-regulate microRNAs (30C and MIR130A) to reduce expression of proteins required for autophagy (ATG5 and ATG16L1) and autophagy response in intestinal epithelial cells. In ileal samples from CD patients, these same microRNAs are augmented and the levels of ATG5 and ATG16L1 diminished[1].

Although AIEC has clearly been related to IBD, whether it is a single etiological invader or just the instigator is not clear. Chassaing *et al*[41] observed that T5KO mice, in early stages of microbiota development inoculated with AIEC, developed colitis that persisted beyond the period in which AIEC could be detected in feces. They observed that AIEC transient colonization altered the gut microbiota of the T5KO mice (not the wild type), by reducing its diversity, which resulted in greater levels of lipopolysaccharides and flagellin that gave the microbiota inherently greater proinflammatory potential, which was responsible for the development of chronic colitis in susceptible hosts. Therefore, the authors concluded from the findings that, in a genetically susceptible host, the presence of a pathobiont (as was AIEC in this case) in a developing microbiota, could result in lasting changes in microbiota composition that might eventuate in chronic inflammation[41]. The question that arises from this excellent study is if the AIEC was not detectable in faeces because it had been cleared or because it was caught in the macrophages and could not be detected.

Regarding urine infections from *E. coli*, fimH mutations were shown to confer significant advantages upon bacteria during bladder colonization in a murine model and to correlate with extraintestinal virulence of *E. coli.* In UPEC, blocking the binding of FimH to its natural receptor prevents bacterial colonization and subsequent inflammation of the urinary tract. Preventive treatments have been developed for UPEC infections: vaccines targeting FimH, mannoside compounds or biarylmannose-derivative FimH antagonists. Similar therapeutic strategies could be useful for preventing AIEC colonization in CD patients[33].

Organisms such as *E. coli* and Salmonellae that express the FimH protein of type 1 pilli have been shown to bind to M cells (microfold cells that overlie Peyer's patches in the intestine and lymphoid follicles in the colon) by interaction between FimH and glycoprotein 2 (GP2), expressed on the apical plasma membrane of M cells; possession of FimH is essential for invasion of M cells by these organisms to occur[42]. It has also been demonstrated that the same GP2 protein is the epitope for the "anti-pancreatic" antibody found in CD sera[43]. This raises the possibility that a combination of bacterial components, including FimH, linked to GP2, may be presented as a foreign antigen and thus lead to development of anti-GP2 antibodies, in a way analogous to the development of anti-tissue transglutaminase antibodies in celiac disease. As Friswell *et al*[44] highlight in their review of the role of bacteria in the pathogenesis of IBD, blockade of bacterial entry *via* M cells represents an important target for therapies. In a very recent paper[45], certain isolates of *E. coli* have been shown to be related both with colorectal cancer and IBD. The authors suggest that interventions that either reduce colonization by diffusely adherent *E. coli* or block their interaction with the mucosa may have preventive or therapeutic effects in colon cancer and CD.

Mycobacterium avium paratuberculosis

Mycobacterium avium subspecies *paratuberculosis* is an obligate pathogenic organism that causes Johne's disease[44] in ruminants and other animals such as primates and rabbits. Johne's disease is a chronic wasting diarrheal disease[18] with clinical and histological conditions that are highly evocative of CD[46]. Like in CD, *Mycobacterium avium paratuberculosis* (MAP) infection causes segmental and fibrosing stenosis, as well as epithelial granulomata[47]. The link between CD and MAP was first postulated by Dalziel in 1913, before Crohn's classic description of CD, when he noted the similarities with Johne's disease.

MAP is historically considered not to be zoonotic, although case reports of infected human beings, with clinically relevant illness, have been published. Humans worldwide are highly exposed to MAP. MAP has been cultured from pasteurized milk, chlorinated potable water, meat products, breast milk from mothers with CD, and from the blood of IBD patients[46,48] and controls.

MAP is quite difficult to culture; it may be present in the cell-wall-deficient form (spheroplasts) and appear negative with Ziehl-Nielsen stain. Under appropriate culture conditions and over a prolonged period (weeks to years), these bacteria produce cell wall and become Ziehl-Nielsen positive. MAP lacks the iron-chelating agent mycobactin, so the infected host or the culture medium must provide the iron for it to grow. Detection of MAP DNA or RNA using PCR is generally preferred because of the shorter time scale and the increased sensitivity of the technique. Early PCR methods have been questioned because of the similarity of the primers for MAP to other non-MAP-mycobacterium which lead to false positives. An IS900-PCR method that gives more

reliable results is currently used. The flaws of the technique, as Greenstein points out, reside in the fact that, in the process of isolating the nucleus to obtain DNA, MAP DNA can be inadvertently removed if it is in the cytoplasm of the infected host (where MAP replicates) and that the presence of MAP DNA is not proof that MAP causes IBD, for it may have coincidentally been ingested without causing infection[18]. According to Greenstein, isolation of MAP RNA indicates that the organism was viable at the time of isolation; it is a technique that is easy to reproduce due to the smaller size of the RNA with respect to DNA, although the half-life of RNA is short (minutes) while DNA may survive for centuries.

In Naser's series[49], MAP was cultured from blood in up to 50% of CD patients and 22% of UC patients, but no control patients. In Mendoza et al's study using CD, UC and controls, MAP DNA was detected in all blood samples. No mycobacterial growth was observed using BACTEC MGTI cultures, but all of the 18-mo cultures from CD patients were positive by phenolic acridine orange staining, which suggested the presence of spheroplasts[15]. All the CD patients and one of the UC patients were observed to have cell-wall-deficient forms, but none of the non-IBD controls contained them.

Two studies carried out in the north of Spain have analyzed the MAP DNA, using IS900 nested PCR, in the blood of IBD and adult control patients. A higher prevalence of MAP infection was observed in healthy individuals than in IBD patients. Elguezabal *et al*[46] found a prevalence of 45.2% MAP DNA in the blood of healthy controls, compared to 21.38% and 19.04% in CD and UC patients respectively. The authors attributed the difference to therapy, as did Juste *et al*[48], who found an even bigger difference between healthy controls (47% MAP DNA in blood) and IBD (16%). Juste *et al*[48] found that 17% of the patients receiving mesalamine, 6% of those taking sulfasalazine, and none of the ones taking methotrexate, mercaptopurine, ciprofloxacin or tacrolimus had MAP DNA detectable in blood; no difference was observed with azathioprine or steroids.

Contrary to the previous finding of a higher MAP DNA prevalence in controls, Kirkwood *et al*[50] found more MAP IS900 DNA and live MAP (culture of gut biopsies) in naïve pediatric CD patients than in non-IBD. The non-IBD patients had lower levels of mucosal IS900 DNA, no MAP in tissue culture and no DNA in blood. The distinguishing characteristic of this study is that the CD patients were naïve, and, therefore, had not received IBD treatment and the disease had not yet evolved.

Another study by Greenstein's group, performed in 1996 in resected intestinal tissue, found RNA of MAP in 100% of the cases. They concluded that, analogous to other mycobacterial infections, such as lepra and tuberculosis, which have different presentations depending on the host's genetic predisposition and immunological state, CD could be secondary to MAP and have two distinct presentations: fistulising and stenosing[51].

An alternative way to study the link between MAP and CD is to assess whether the CD patients have MAP antibodies that react to MAP antigens. Meta-analyses have shown reactivity in CD patients' sera, to MAP p35 and p36 recombinant antigens. However, it should be noted that MAP p35 and p36 are similar to that of *Mycobacterium avium*, subspecies *avium [MAA]*, so a specific reaction to MAP rather than MAA cannot be certain[44]. An increased presence of MAP-reactive T cells has also been found in CD patients but not in controls[52].

CD patients show increased levels of antibodies against saccharomyces cerevisiae (ASCA), the epitope of which is a mannose which is present in yeast walls. Studies have demonstrated that not only is MAP a possible source for the ASCA mannan epitope, but also that MAP release a mannose-containing glycoconjugate that impairs the *in vitro* ability of monocyte-derived macrophages to kill phagocytosed *E. coli*. Therefore, as Friswell suggests, MAP might be acting *via* an indirect pathogenic effect, which would explain its role in pathogenesis and yet not be greatly exacerbated by anti-TNF treatment[44,53]. ASCA may develop a long time before the diagnosis of CD is established. ASCA have a genetically modulated expression, found in 20%-25% of the relatives of CD patients, and not in their spouses[54].

Several studies highlight how genetic alterations found in CD can favor MAP infection. Ferwerda *et al*[55] demonstrated that NOD2 mutant patients show an ineffective recognition of MAP. Hansen *et al*[4]'s review explains that Gutierrez *et al*[56] showed that defective ATG16L1 function (which is found in CD patients) avoids *Mycobacterium tuberculosis* inhibition in macrophages, supporting the idea of a *Mycobacterial* pathogen in IBD[4,56]. Finally, Sechi *et al*[57] found a relationship between CD, MAP infection and SLC11A1 gene polymorphisms.

One of the puzzling aspects of MAP as a causative agent was that IBD treatments were not worsening a possible MAP infection. To answer this question, Greenstein's group published several studies in which they demonstrated that numerous IBD-drugs inhibited MAP growth *in vitro*: 5-ASA, mercaptopurine, methotrexate, cyclosporine, rapamycine and tacrolimus, most acting in a dose-dependent manner. The authors suggested that the medical profession had been treating MAP infections mistakenly since the introduction of sulfasalazine in 1942. This is supported by results like Juste's *et al*'s, in which no DNA is found in peripheral blood of IBD patients treated with immunosuppressants.

An argument against MAP being clinically relevant in IBD is that antibiotic treatment does not cure

IBD. The authors who favor MAP as causative agent remark that most IBD antibiotic regimens are not fully effective for MAP and that good MAP treatments need to include macrolides, should include triple or quadruple therapies, or should be sustained for very long periods of time, as is done in tuberculosis treatment. Several long-term regimens have been performed, showing initial improvement, with steroid weaning, but concluding that the benefit is not sustained[58,59]. A two-year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for CD was used in a study performed in Australia, in which significant improvement up to week 16 was observed, although subsequent follow-up results were not conclusive. However, correspondence in a Gastroenterology Journal in 2007 pointed out that less benefit was obtained with other IBD therapies and that none achieved uniform long-term sustained remission.

Greenstein and his group have frequently stated that MAP has met Koch's four postulates, *i.e.*, it is found in tissue of CD patients, can be cultured, can reproduce the disease when inoculated into other animals and can be re-isolated from the diseased subject. A paper by Momotani, among others, supported this idea by proving that MAP could cause CD-type necrotizing colitis in mice. However, as we have previously seen, MAP DNA can also be found in healthy controls, sometimes in higher proportions than in IBD patients. Whether this is a consequence of exposure and not infection, or if IBD treatment inhibits MAP but does not clear it up, or if MAP acts as a co-causative agent by impairing killing of bacteria, such as *E. coli*, all remain to be elucidated.

Helicobacter pylori

Several studies have postulated that *Helicobacter pylori* (HP) infection has a protective role against chronic inflammatory diseases, like IBD. These studies based their statements on the laboratory results that proved that HP could induce immune tolerance, limiting the inflammatory response[60]. Some preliminary studies suggested that patients infected with HP probably had less risk of developing IBD than the rest of the general population. The mechanisms for decreased prevalence of HP in IBD were not ascertained[61].

In 2001, Väre *et al*[62] published a study with 296 patients: 185 had UC, 94 CD and 17 had indeterminate colitis (IC). HP antibodies were determined. The results confirmed the low prevalence of HP infection, especially in CD patients. Age of onset of IBD was higher in seropositive (mean 40 years) than in seronegative patients. The age of onset of IBD showed unimodal distribution in *H. pylori* seronegative patients, with a peak between 30 and 40 years. In contrast, *H. pylori* seropositive patients showed a clear bimodal pattern with peaks at 20-40 and 50-60 years of age. The results suggested that HP could significantly modify the appearance of IBD, perhaps with a protective effect[62].

In 2010, Luther *et al*[60] carried out an extensive review of the papers published on HP, as well as a meta-analysis that evaluated the possible relationship between IBD and the presence or lack of HP infection. Five Thousand and ninety-three patients were evaluated. Twenty-seven percent of the IBD patients had HP infection, in comparison to 40% of the control group, with an estimated relative risk of infection of 0.64%. These results could suggest a protective role, but the authors emphasized that the heterogeneity in the studies included could not be accounted for by the method of IBD and *H. pylori* diagnosis, study location, or study population age, which limited the value of the results[60].

In 2011, the same authors published a study that tried to clarify the mechanism responsible for the inverse association of HP and IBD. The authors first assumed the postulate that bacterial DNA in distal intestine could influence mucosal immunity. Several papers have documented that HP DNA can be found both in the colon and feces of infected patients. The authors demonstrated that, contrary to what DNA from other bacteria such as *E. coli* do, which induce an inflammatory response from dendritic cells *in vitro*, HP DNA is unable to induce inflammatory reaction. On the other hand, it is capable of inhibiting the production of proinflammatory cytokines of the murine or human cells *in vitro*. They also demonstrated that HP infected patients have lower systemic levels of type Ⅰ IFN, in comparison to uninfected patients. The authors concluded that the inverse correlation between IBD and HP, observed in epidemiological studies, could be partially explained by HP DNA's capacity to reduce type Ⅰ IFN[61].

More recently, in 2012 Sonnenberg *et al*[63,64], carried out a study in which they performed upper and lower endoscopy (on the same day) to a large sample of patients. Biopsies obtained in the upper endoscopy were examined for esophagitis, gastritis and HP infection. Biopsies obtained from colonoscopy were scrutinized for UC, CD and IC. IBD was identified in 1061 patients (1.6%), and the rest was used as control group. The analysis showed an inverse relationship between HP and IBD, both for UC and CD. On the other hand, a positive relationship was found between HP-negative chronic gastritis and IBD[63,64].

Data obtained in the different types of studies performed to date (epidemiological, clinical and experimental) suggest that HP infection reduces the risk of developing IBD, by producing a protective effect that hinders the appearance of IBD. Case reports and epidemiological studies have observed that IBD may appear quickly after HP eradication, possibly due to a shift in the Th pattern. However, other studies have exposed that this lower preva-

lence of HP in IBD might be secondary to HP "spontaneous eradication" with 5-ASA or antibiotic treatment[65].

Other Helicobacter species, like *Helicobacter hepaticus*, have shown their capability of inducing colitis in animals, mainly in immunocompromised ones. Studies need to be done to see if the same effect is seen in humans[9,66,67].

Clostridium difficile

Clostridium difficile (*C. Difficile*) is a gram positive anaerobic bacillus that forms spores and produces toxins, which can cause different degrees of intestinal disease. Classically, it has been considered a cause of colitis related to the use of antibiotics and of nosocomial diarrhea. In the last decades, its incidence has greatly increased. Nowadays, 20%-30% of antibiotic-related diarrheas and up to 50%-70% of the antibiotic-related colitis are considered to be due to *C. Difficile* infection. A similar increase in the incidence of *C. Difficile* infection in IBD has been documented. Up to 10% of the IBD patients will develop *C. Difficile* infection at some point. IBD patients probably have a higher risk of infections due to increased antibiotic use and the consequent creation of a favorable environment for colonization, although in 40% of the cases in IBD patients, it can appear without previous use of antibiotic. Possible complications in these patients include colectomy and death. However, there are no conclusive data in the medical literature to support the idea of the bacteria as a cause of onset or reactivation of IBD, which could be merely a consequence of the existing inflammatory status[68,69].

As mentioned, many studies have observed an increased risk of *C. Difficile* infection in IBD, which may be due to a variety of factors that include the altered nutritional and immunological state, repeated hospitalizations, frequent and recurrent use of antibiotics and immunomodulators and even genetic predisposition. Although *C. Difficile* may not be a causative agent, it can produce superimposed colitis or induce reactivation, and, therefore, is nowadays considered a risk factor of exacerbations.

In 2013, Nitzan *et al*[70] published an extensive review regarding the role of *C. Difficile* in the pathogenesis of IBD, as well as its implications with respect to diagnosis and treatment. The review embraces the different risk factors, clinical characteristics of the infection in IBD, special aspects of its presentation, diagnosis and treatment in IBD. The authors emphasize the necessary suspicion of diagnosis, recommending screening in every IBD patient hospitalized for a flare, as well as early treatment, especially in severe cases. They conclude that *Clostridium Difficile* most likely plays a role in the pathogenesis of exacerbations, although probably not in the development of IBD itself[70].

Campylobacter and salmonella

A recent study by Jess *et al*[71] analyzed the incidence of CD and UC in patients with positive and negative fecal *Salmonella* and *Campylobacter* tests, as well as the incidence of positive and negative cultures in those already diagnosed with IBD. To do so, the researchers analyzed the patients that had been included in the Danish national register in the previous 15 years, who had a positive or negative stool test for Salmonella and Campylobacter, and patients diagnosed with IBD[71]. Statistical analysis showed that the risk of developing IBD is relatively high (RR = of 5.4-9.8) the first year after a positive fecal test for Salmonella or Campylobacter. It remains moderately high up to 10 years after the positive test (RR = 1.6-2.2) and becomes low after 10 years (0.8-1.8).

However, the first year after a negative stool test the relative risk of IBD was also high, and a decreasing incidence pattern over time was parallel to that following positive test results.

The risk of having IBD substantially increases, not only after an infection with Salmonella or Campylobacter, but also, and even more so, after a patient has a negative stool test. After 10 years of positive results, the relative risk of developing IBD is reduced to 1, but the risk remains quite high 10 years after having a stool test, in the case that the test had been negative. During the first year after a first hospitalization for IBD, the risk of a negative test is high and remains high.

In conclusion, the study confirmed the previous results of a higher risk of IBD after *Salmonella* or *Campylobacter* infection[72], but it revealed that the risk is perhaps surprisingly more pronounced after a negative stool test. These results might suggest that the risk which had previously been attributed to *Salmonella* and *Campylobacter* gastroenteritis could in fact be due to the fact that more tests are carried out, rather than to a casual effect.

ROLE OF VIRUSES IN THE ETIOLOGY OF IBD

The role of the different viruses in the pathogenesis of IBD is still not well understood. Two theories, based on many epidemiological studies with contradictory results, have been proposed to explain the relationship between viral infections and the development of IBD, which, in part, may be mutually exclusive. The first theory suggests that certain infections that occur during infancy may predispose to the appearance of IBD. The second theory points to the absence of infections in infancy and the lack of contact with certain antigens as the cause of subsequent intestinal inflammation (Hygiene Theory).

An example that supports infections as a risk

factor is found in Ekbom's case-control study, carried out in 1990 to determine the potential role of infections during infancy in the pathogenesis of IBD[73]. Analysis of perinatal events and risk of CD found postnatal infections to be the factor that was associated most strongly, both in the univariant analysis (OR = 9.5) and the multivariant (OR = 5.5). On the other hand, the fact that IBD appears more frequently in developed countries or in people who migrate from undeveloped to developed countries sustains the hygiene theory as the main etiological factor.

Measles

The possible role of the measles virus was strengthened in the 1990's by the epidemiological studies that showed the incidence of CD, which was higher than expected, in children that had been born in the three months after measles outbreaks[74,75]. These studies observed that the virus was capable of producing an inflammatory reaction in the mesenteric endothelium that was quite similar to the one found in CD[75-77]. This finding stimulated the performance of numerous observational studies, which analyzed the relationship between measles infection or vaccination and the development of IBD. The studies, which in general were case-control or cohort, obtained heterogeneous results[78-85]. Some had limitations in data gathering and do not enable confirmation of an epidemiological relationship between measles virus and IBD.

A reasonable argument against the relationship between measles and IBD could be the increasing incidence of CD despite the progressive reduction of the measles virus infection. The increase could be due to a change in the virulence factors of the microorganism, as a consequence of widespread vaccination. The question arose if the effect of the attenuated vaccine was responsible for the increase in CD incidence, but again no relationship was found[79,81].

Several later attempts to isolate virus in tissue or blood with more specific techniques were unsuccessfu[86]; the possibility of immunological cross reaction between intestinal or viral antigens was also discarded[87-89]. Consequently, since the minimum data necessary to establish a causal biological relationship has not been attained, the implication of the measles virus or its vaccine[90] in the pathogenesis of IBD is uncertain.

Mumps (parotiditis)

Similarly, during the same period of time, the hypothesis of the parotiditis virus as a pathogenic agent in IBD was proposed. Several studies were published evaluating the epidemiological association between mumps infection and IBD, both alone or with measles co-infection. Although some identified a higher IBD risk[91,92], viral parts were not isolated from intestinal tissue[93] and the direct link with IBD could not be established. While it was suggested that the immunological response to the virus might be involved in the process of IBD presentation, as was observed with the measles virus, no concluding evidence was found[94].

Citomegalovirus

The role of Citomegalovirus (CMV) as an infectious agent that can reactivate underlying inflammatory disease has been accepted[95]. However, there is no proof of a CMV effect on the pathogenesis. Despite the association between the intensity of expression in the intestinal mucosa and the severity of the IBD inflammation, viral replication was also found in healthy mucosa[96]. A higher CMV infection rate was not observed in the IBD population with respect to the general population[97].

Virus de Epstein-Barr

Interest in a potential etiopathogenic role of Virus de Epstein-Barr (VEB) in IBD arose when an increased number of B lymphocytes infected with VEB was found in mucosal samples from UC colons, and, to a lesser degree, in CD samples[98]. Epidemiological data indicate that frequency of infection is similar in IBD patients and healthy controls, both approximately 100%[99]. The intensity of replication has been related to increased bowel inflammation[100] and to serious complications such as lymphomas[95,101]. Therefore, as with CMV, a modulating function, rather than an etiological implication, was determined.

CONCLUSION

The significant role of microorganisms in the pathogenesis of IBD seems apparent, although it is complex. Whether it is one pathogenic germ or more than one in unison with the gut microbiota as helper, main character or observer needs to be elucidated. Clinical suspicion, now widely supported by genetic and molecular studies, points to altered autophagy that may favor intracellular germs that trigger a cascade of events that produce the onset of IBD in predisposed individuals. Studies now implicating AIEC as pivotal in CD, mainly ileal CD, raise the question of if we have been discarding one of the main players, *E. coli*, as crucial because it was considered microbiota, without understanding its more adherent and invasive particularities in IBD patients and if the same is the case with other intestinal "bugs". Or is CD caused, as happens with MAP, by chronic exposure to a slow-acting germ? Or are we simply in a "bacteria era" which will pass, as did the "virus era", when more information is obtained and understood regarding the pathogenesis of CD? Many questions remain. Understanding the interaction of several germs together, as seen with *E. coli* and MAP, which may, in conjunction favor IBD, or

with HP, which may keep the balance away from IBD, might clarify the base of this complex disorder.

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REFERENCES

1 **Nguyen HT**, Dalmasso G, Müller S, Carrière J, Seibold F, Darfeuille-Michaud A. Crohn's disease-associated adherent invasive Escherichia coli modulate levels of microRNAs in intestinal epithelial cells to reduce autophagy. *Gastroenterology* 2014; **146**: 508-519 [PMID: 24148619]

2 **Kaser A**, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol* 2010; **28**: 573-621 [PMID: 20192811 DOI: 10.1146/annurev-immunol-030409-101225]

3 **Leone V**, Chang EB, Devkota S. Diet, microbes, and host genetics: the perfect storm in inflammatory bowel diseases. *J Gastroenterol* 2013; **48**: 315-321 [PMID: 23475322 DOI: 10.1007/s00535-013-0777-2]

4 **Hansen R**, Thomson JM, El-Omar EM, Hold GL. The role of infection in the aetiology of inflammatory bowel disease. *J Gastroenterol* 2010; **45**: 266-276 [PMID: 20076977 DOI: 10.1007/s00535-009-0191-y]

5 **Cani PD**, Delzenne NM. The gut microbiome as therapeutic target. *Pharmacol Ther* 2011; **130**: 202-212 [PMID: 21295072 DOI: 10.1016/j.pharmthera.2011.01.012]

6 **Cave DR**, Mitchell DN, Brooke BN. Experimental animal studies of the etiology and pathogenesis of Crohn's disease. *Gastroenterology* 1975; **69**: 618-624 [PMID: 1158079]

7 **Cave DR**, Mitchell DN, Kane SP, Brooke BN. Further animal evidence of a transmissible agent in Crohn's disease. *Lancet* 1973; **2**: 1120-1122 [PMID: 4128014]

8 **Mitchell DN**, Rees RJ, Goswami KK. Transmissible agents from human sarcoid and Crohn's disease tissues. *Lancet* 1976; **2**: 761-765 [PMID: 61441]

9 **Kullberg MC**, Ward JM, Gorelick PL, Caspar P, Hieny S, Cheever A, Jankovic D, Sher A. Helicobacter hepaticus triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. *Infect Immun* 1998; **66**: 5157-5166 [PMID: 9784517]

10 **Sewell GW**, Marks DJ, Segal AW. The immunopathogenesis of Crohn's disease: a three-stage model. *Curr Opin Immunol* 2009; **21**: 506-513 [PMID: 19665880 DOI: 10.1016/j.coi.2009.06.003]

11 **Chassaing B**, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 2011; **140**: 1720-1728 [PMID: 21530738 DOI: 10.1053/j.gastro.2011.01.054]

12 **Manichanh C**, Borruel N, Casellas F, Guarner F. The gut microbiota in IBD. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 599-608 [PMID: 22907164 DOI: 10.1038/nrgastro.2012.152]

13 **Hudson MJ**, Hill MJ, Elliott PR, Berghouse LM, Burnham WR, Lennard-Jones JE. The microbial flora of the rectal mucosa and faeces of patients with Crohn's disease before and during antimicrobial chemotherapy. *J Med Microbiol* 1984; **18**: 335-345 [PMID: 6334160]

14 **Mendoza JL**, Lana R, Díaz-Rubio M. Mycobacterium avium subspecies paratuberculosis and its relationship with Crohn' s disease. *World J Gastroenterol* 2009; **15**: 417-422 [PMID: 19152445]

15 **Mendoza JL**, San-Pedro A, Culebras E, Cíes R, Taxonera C, Lana R, Urcelay E, de la Torre F, Picazo JJ, Díaz-Rubio M. High prevalence of viable Mycobacterium avium subspecies paratuberculosis in Crohn's disease. *World J Gastroenterol* 2010; **16**: 4558-4563 [PMID: 20857526]

16 **Momotani E**, Romona NM, Yoshihara K, Momotani Y, Hori M, Ozaki H, Eda S, Ikegami M. Molecular pathogenesis of bovine paratuberculosis and human inflammatory bowel diseases. *Vet Immunol Immunopathol* 2012; **148**: 55-68 [PMID: 22486997 DOI: 10.1016/j.vetimm.2012.03.005]

17 **Greenstein RJ**, Su L, Juste RA, Brown ST. On the action of cyclosporine A, rapamycin and tacrolimus on M. avium including subspecies paratuberculosis. *PLoS One* 2008; **3**: e2496 [PMID: 18575598 DOI: 10.1371/journal.pone.0002496]

18 **Greenstein RJ**. Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease. *Lancet Infect Dis* 2003; **3**: 507-514 [PMID: 12901893]

19 **Darfeuille-Michaud A**, Neut C, Barnich N, Lederman E, Di Martino P, Desreumaux P, Gambiez L, Joly B, Cortot A, Colombel JF. Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn's disease. *Gastroenterology* 1998; **115**: 1405-1413 [PMID: 9834268]

20 **Boudeau J**, Glasser AL, Masseret E, Joly B, Darfeuille-Michaud A. Invasive ability of an Escherichia coli strain isolated from the ileal mucosa of a patient with Crohn's disease. *Infect Immun* 1999; **67**: 4499-4509 [PMID: 10456892]

21 **Glasser AL**, Boudeau J, Barnich N, Perruchot MH, Colombel JF, Darfeuille-Michaud A. Adherent invasive Escherichia coli strains from patients with Crohn's disease survive and replicate within macrophages without inducing host cell death. *Infect Immun* 2001; **69**: 5529-5537 [PMID: 11500426]

22 **Masseret E**, Boudeau J, Colombel JF, Neut C, Desreumaux P, Joly B, Cortot A, Darfeuille-Michaud A. Genetically related Escherichia coli strains associated with Crohn's disease. *Gut* 2001; **48**: 320-325 [PMID: 11171820]

23 **Darfeuille-Michaud A**, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, Bringer MA, Swidsinski A, Beaugerie L, Colombel JF. High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. *Gastroenterology* 2004; **127**: 412-421 [PMID: 15300573]

24 **Rutgeerts P**, Goboes K, Peeters M, Hiele M, Penninckx F, Aerts R, Kerremans R, Vantrappen G. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. *Lancet* 1991; **338**: 771-774 [PMID: 1681159]

25 **O'Brien CL**, Pavli P, Gordon DM, Allison GE. Detection of bacterial DNA in lymph nodes of Crohn's disease patients using high throughput sequencing. *Gut* 2014; **63**: 1596-1606 [PMID: 24429583 DOI: 10.1136/gutjnl-2013-305320]

26 **Baumgart M**, Dogan B, Rishniw M, Weitzman G, Bosworth B, Yantiss R, Orsi RH, Wiedmann M, McDonough P, Kim SG, Berg D, Schukken Y, Scherl E, Simpson KW. Culture independent analysis of ileal mucosa reveals a selective increase in invasive Escherichia coli of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *ISME J* 2007; **1**: 403-418 [PMID: 18043660]

27 **Nataro JP**, Kaper JB. Diarrheagenic Escherichia coli. *Clin Microbiol Rev* 1998; **11**: 142-201 [PMID: 9457432]

28 **Cartun RW**, Van Kruiningen HJ, Pedersen CA, Berman MM. An immunocytochemical search for infectious agents in Crohn's disease. *Mod Pathol* 1993; **6**: 212-219 [PMID: 8483893]

29 **Tabaqchali S**, O'Donoghue DP, Bettelheim KA. Escherichia coli antibodies in patients with inflammatory bowel disease. *Gut* 1978; **19**: 108-113 [PMID: 344155]

30 **Mann EA**, Saeed SA. Gastrointestinal infection as a trigger for inflammatory bowel disease. *Curr Opin Gastroenterol* 2012; **28**: 24-29 [PMID: 22080823 DOI: 10.1097/MOG.0b013e32834c453e]

31 **Barnich N**, Darfeuille-Michaud A. Role of bacteria in the etiopathogenesis of inflammatory bowel disease. *World J Gastroenterol* 2007; **13**: 5571-5576 [PMID: 17948930]

32 **Wine E**, Ossa JC, Gray-Owen SD, Sherman PM. Adherent-invasive Escherichia coli target the epithelial barrier. *Gut Microbes* 2010; **1**: 80-84 [PMID: 21326914]

33 **Dreux N**, Denizot J, Martinez-Medina M, Mellmann A, Billig M, Kisiela D, Chattopadhyay S, Sokurenko E, Neut C, Gower-Rousseau C, Colombel JF, Bonnet R, Darfeuille-Michaud A, Barnich N. Point mutations in FimH adhesin of Crohn's disease-

associated adherent-invasive Escherichia coli enhance intestinal inflammatory response. *PLoS Pathog* 2013; **9**: e1003141 [PMID: 23358328 DOI: 10.1371/journal.ppat.1003141]

34 **Moulin-Schouleur M**, Répérant M, Laurent S, Brée A, Mignon-Grasteau S, Germon P, Rasschaert D, Schouler C. Extraintestinal pathogenic Escherichia coli strains of avian and human origin: link between phylogenetic relationships and common virulence patterns. *J Clin Microbiol* 2007; **45**: 3366-3376 [PMID: 17652485]

35 **Wirth T**, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M. Sex and virulence in Escherichia coli: an evolutionary perspective. *Mol Microbiol* 2006; **60**: 1136-1151 [PMID: 16689791]

36 **Miquel S**, Peyretaillade E, Claret L, de Vallée A, Dossat C, Vacherie B, Zineb el H, Segurens B, Barbe V, Sauvanet P, Neut C, Colombel JF, Medigue C, Mojica FJ, Peyret P, Bonnet R, Darfeuille-Michaud A. Complete genome sequence of Crohn's disease-associated adherent-invasive E. coli strain LF82. *PLoS One* 2010; **5**: e12714 [PMID: 20862302 DOI: 10.1371/journal.pone.0012714]

37 **Rolhion N**, Barnich N, Bringer MA, Glasser AL, Ranc J, Hébuterne X, Hofman P, Darfeuille-Michaud A. Abnormally expressed ER stress response chaperone Gp96 in CD favours adherent-invasive Escherichia coli invasion. *Gut* 2010; **59**: 1355-1362 [PMID: 20587550 DOI: 10.1136/gut.2010.207456]

38 **Chassaing B**, Rolhion N, de Vallée A, Salim SY, Prorok-Hamon M, Neut C, Campbell BJ, Söderholm JD, Hugot JP, Colombel JF, Darfeuille-Michaud A. Crohn disease--associated adherent-invasive E. coli bacteria target mouse and human Peyer's patches via long polar fimbriae. *J Clin Invest* 2011; **121**: 966-975 [PMID: 21339647]

39 **Lapaquette P**, Glasser AL, Huett A, Xavier RJ, Darfeuille-Michaud A. Crohn's disease-associated adherent-invasive E. coli are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol* 2010; **12**: 99-113 [PMID: 19747213 DOI: 10.1111/j.1462-5822.2009.01381]

40 **Matricon J**, Barnich N, Ardid D. Immunopathogenesis of inflammatory bowel disease. *Self Nonself* 2010; **1**: 299-309 [PMID: 21487504]

41 **Chassaing B**, Koren O, Carvalho FA, Ley RE, Gewirtz AT. AIEC pathobiont instigates chronic colitis in susceptible hosts by altering microbiota composition. *Gut* 2014; **63**: 1069-1080 [PMID: 23896971 DOI: 10.1136/gutjnl-2013-304909]

42 **Hase K**, Kawano K, Nochi T, Pontes GS, Fukuda S, Ebisawa M, Kadokura K, Tobe T, Fujimura Y, Kawano S, Yabashi A, Waguri S, Nakato G, Kimura S, Murakami T, Iimura M, Hamura K, Fukuoka S, Lowe AW, Itoh K, Kiyono H, Ohno H. Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. *Nature* 2009; **462**: 226-230 [PMID: 19907495 DOI: 10.1038/nature08529]

43 **Roggenbuck D**, Hausdorf G, Martinez-Gamboa L, Reinhold D, Büttner T, Jungblut PR, Porstmann T, Laass MW, Henker J, Büning C, Feist E, Conrad K. Identification of GP2, the major zymogen granule membrane glycoprotein, as the autoantigen of pancreatic antibodies in Crohn's disease. *Gut* 2009; **58**: 1620-1628 [PMID: 19549613 DOI: 10.1136/gut.2008.162495]

44 **Friswell M**, Campbell B, Rhodes J. The role of bacteria in the pathogenesis of inflammatory bowel disease. *Gut Liver* 2010; **4**: 295-306 [PMID: 20981205 DOI: 10.5009/gnl.2010.4.3.295]

45 **Prorok-Hamon M**, Friswell MK, Alswied A, Roberts CL, Song F, Flanagan PK, Knight P, Codling C, Marchesi JR, Winstanley C, Hall N, Rhodes JM, Campbell BJ. Colonic mucosa-associated diffusely adherent afaC+ Escherichia coli expressing lpfA and pks are increased in inflammatory bowel disease and colon cancer. *Gut* 2014; **63**: 761-770 [PMID: 23846483 DOI: 10.1136/gutjnl-2013-304739]

46 **Elguezabal N**, Chamorro S, Molina E, Garrido JM, Izeta A, Rodrigo L, Juste RA. Lactase persistence, NOD2 status and Mycobacterium avium subsp. paratuberculosis infection associations to Inflammatory Bowel Disease. *Gut Pathog* 2012; **4**: 6 [PMID: 22742424 DOI: 10.1186/1757-4749-4-6]

47 **Lidar M**, Langevitz P, Shoenfeld Y. The role of infection in inflammatory bowel disease: initiation, exacerbation and protection. *Isr Med Assoc J* 2009; **11**: 558-563 [PMID: 19960852]

48 **Juste RA**, Elguezabal N, Garrido JM, Pavon A, Geijo MV, Sevilla I, Cabriada JL, Tejada A, García-Campos F, Casado R, Ochotorena I, Izeta A, Greenstein RJ. On the prevalence of M. avium subspecies paratuberculosis DNA in the blood of healthy individuals and patients with inflammatory bowel disease. *PLoS One* 2008; **3**: e2537 [PMID: 18596984 DOI: 10.1371/journal.pone.0002537]

49 **Naser SA**, Ghobrial G, Romero C, Valentine JF. Culture of Mycobacterium avium subspecies paratuberculosis from the blood of patients with Crohn's disease. *Lancet* 2004; **364**: 1039-1044 [PMID: 15380962]

50 **Kirkwood CD**, Wagner J, Boniface K, Vaughan J, Michalski WP, Catto-Smith AG, Cameron DJ, Bishop RF. Mycobacterium avium subspecies paratuberculosis in children with early-onset Crohn's disease. *Inflamm Bowel Dis* 2009; **15**: 1643-1655 [PMID: 19462429 DOI: 10.1002/ibd.20967]

51 **Mishina D**, Katsel P, Brown ST, Gilberts EC, Greenstein RJ. On the etiology of Crohn disease. *Proc Natl Acad Sci USA* 1996; **93**: 9816-9820 [PMID: 8790414]

52 **Olsen I**, Tollefsen S, Aagaard C, Reitan LJ, Bannantine JP, Andersen P, Sollid LM, Lundin KE. Isolation of Mycobacterium avium subspecies paratuberculosis reactive CD4 T cells from intestinal biopsies of Crohn's disease patients. *PLoS One* 2009; **4**: e5641 [PMID: 19479064 DOI: 10.1371/journal.pone.0005641]

53 **Mpofu CM**, Campbell BJ, Subramanian S, Marshall-Clarke S, Hart CA, Cross A, Roberts CL, McGoldrick A, Edwards SW, Rhodes JM. Microbial mannan inhibits bacterial killing by macrophages: a possible pathogenic mechanism for Crohn's disease. *Gastroenterology* 2007; **133**: 1487-1498 [PMID: 17919633]

54 **Tsianos EV**, Katsanos K. Do we really understand what the immunological disturbances in inflammatory bowel disease mean? *World J Gastroenterol* 2009; **15**: 521-525 [PMID: 19195052]

55 **Ferwerda G**, Kullberg BJ, de Jong DJ, Girardin SE, Langenberg DM, van Crevel R, Ottenhoff TH, Van der Meer JW, Netea MG. Mycobacterium paratuberculosis is recognized by Toll-like receptors and NOD2. *J Leukoc Biol* 2007; **82**: 1011-1018 [PMID: 17652449]

56 **Gutierrez MG**, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell* 2004; **119**: 753-766 [PMID: 15607973]

57 **Sechi LA**, Gazouli M, Sieswerda LE, Molicotti P, Ahmed N, Ikonomopoulos J, Scanu AM, Paccagnini D, Zanetti S. Relationship between Crohn's disease, infection with Mycobacterium avium subspecies paratuberculosis and SLC11A1 gene polymorphisms in Sardinian patients. *World J Gastroenterol* 2006; **12**: 7161-7164 [PMID: 17131479]

58 **Gui GP**, Thomas PR, Tizard ML, Lake J, Sanderson JD, Hermon-Taylor J. Two-year-outcomes analysis of Crohn's disease treated with rifabutin and macrolide antibiotics. *J Antimicrob Chemother* 1997; **39**: 393-400 [PMID: 9096189]

59 **Selby W**, Pavli P, Crotty B, Florin T, Radford-Smith G, Gibson P, Mitchell B, Connell W, Read R, Merrett M, Ee H, Hetzel D. Two-year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for Crohn's disease. *Gastroenterology* 2007; **132**: 2313-2319 [PMID: 17570206]

60 **Luther J**, Dave M, Higgins PD, Kao JY. Association between Helicobacter pylori infection and inflammatory bowel disease: a meta-analysis and systematic review of the literature. *Inflamm Bowel Dis* 2010; **16**: 1077-1084 [PMID: 19760778 DOI: 10.1002/ibd.21116]

61 **Luther J**, Owyang SY, Takeuchi T, Cole TS, Zhang M, Liu M, Erb-Downward J, Rubenstein JH, Chen CC, Pierzchala AV, Paul JA, Kao JY. Helicobacter pylori DNA decreases pro-inflammatory cytokine production by dendritic cells and attenuates dextran sodium sulphate-induced colitis. *Gut* 2011; **60**: 1479-1486 [PMID: 21471567 DOI: 10.1136/gut.2010.220087]

62 **Väre PO**, Heikius B, Silvennoinen JA, Karttunen R, Niemelä SE, Lehtola JK, Karttunen TJ. Seroprevalence of Helicobacter pylori

infection in inflammatory bowel disease: is Helicobacter pylori infection a protective factor? *Scand J Gastroenterol* 2001; **36**: 1295-1300 [PMID: 11761020]
63 **Sonnenberg A**, Genta RM. Low prevalence of Helicobacter pylori infection among patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2012; **35**: 469-476 [PMID: 22221289 DOI: 10.1111/j.1365-2036.2011.04969]
64 **Sonnenberg A**, Melton SD, Genta RM. Frequent occurrence of gastritis and duodenitis in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2011; **17**: 39-44 [PMID: 20848539 DOI: 10.1002/ibd.21356]
65 **Papamichael K**, Konstantopoulos P, Mantzaris GJ. Helicobacter pylori infection and inflammatory bowel disease: is there a link? *World J Gastroenterol* 2014; **20**: 6374-6385 [PMID: 24914359]
66 **Kullberg MC**, Jankovic D, Feng CG, Hue S, Gorelick PL, McKenzie BS, Cua DJ, Powrie F, Cheever AW, Maloy KJ, Sher A. IL-23 plays a key role in Helicobacter hepaticus-induced T cell-dependent colitis. *J Exp Med* 2006; **203**: 2485-2494 [PMID: 17030948]
67 **Morrison PJ**, Bending D, Fouser LA, Wright JF, Stockinger B, Cooke A, Kullberg MC. Th17-cell plasticity in Helicobacter hepaticus-induced intestinal inflammation. *Mucosal Immunol* 2013; **6**: 1143-1156 [PMID: 23462910]
68 **Bien J**, Palagani V, Bozko P. The intestinal microbiota dysbiosis and Clostridium difficile infection: is there a relationship with inflammatory bowel disease? *Therap Adv Gastroenterol* 2013; **6**: 53-68 [PMID: 23320050 DOI: 10.1177/1756283X12454590]
69 **Navaneethan U**, Venkatesh PG, Shen B. Clostridium difficile infection and inflammatory bowel disease: understanding the evolving relationship. *World J Gastroenterol* 2010; **16**: 4892-4904 [PMID: 20954275]
70 **Nitzan O**, Elias M, Chazan B, Raz R, Saliba W. Clostridium difficile and inflammatory bowel disease: role in pathogenesis and implications in treatment. *World J Gastroenterol* 2013; **19**: 7577-7585 [PMID: 24282348]
71 **Jess T**, Simonsen J, Nielsen NM, Jørgensen KT, Bager P, Ethelberg S, Frisch M. Enteric Salmonella or Campylobacter infections and the risk of inflammatory bowel disease. *Gut* 2011; **60**: 318-324 [PMID: 21193449 DOI: 10.1136/gut.2010.223396]
72 **Gradel KO**, Nielsen HL, Schønheyder HC, Ejlertsen T, Kristensen B, Nielsen H. Increased short- and long-term risk of inflammatory bowel disease after salmonella or campylobacter gastroenteritis. *Gastroenterology* 2009; **137**: 495-501 [PMID: 19361507 DOI: 10.1053/j.gastro.2009.04.001]
73 **Ekbom A**, Adami HO, Helmick CG, Jonzon A, Zack MM. Perinatal risk factors for inflammatory bowel disease: a case-control study. *Am J Epidemiol* 1990; **132**: 1111-1119 [PMID: 2260543]
74 **Ekbom A**, Wakefield AJ, Zack M, Adami HO. Perinatal measles infection and subsequent Crohn's disease. *Lancet* 1994; **344**: 508-510 [PMID: 7914614]
75 **Wakefield AJ**, Pittilo RM, Sim R, Cosby SL, Stephenson JR, Dhillon AP, Pounder RE. Evidence of persistent measles virus infection in Crohn's disease. *J Med Virol* 1993; **39**: 345-353 [PMID: 8492105]
76 **Wakefield AJ**, Ekbom A, Dhillon AP, Pittilo RM, Pounder RE. Crohn's disease: pathogenesis and persistent measles virus infection. *Gastroenterology* 1995; **108**: 911-916 [PMID: 7875495]
77 **Wakefield AJ**, Sim R, Akbar AN, Pounder RE, Dhillon AP. In situ immune responses in Crohn's disease: a comparison with acute and persistent measles virus infection. *J Med Virol* 1997; **51**: 90-100 [PMID: 9021538]
78 **Van Damme W**, Lynen L, Kegels G, Van Lerberghe W. Measles vaccination and inflammatory bowel disease. *Lancet* 1997; **350**: 1774-1775 [PMID: 9413483]
79 **Thompson NP**, Montgomery SM, Pounder RE, Wakefield AJ. Is measles vaccination a risk factor for inflammatory bowel disease? *Lancet* 1995; **345**: 1071-1074 [PMID: 7715338]
80 **Morris DL**, Montgomery SM, Thompson NP, Ebrahim S, Pounder RE, Wakefield AJ. Measles vaccination and inflammatory bowel disease: a national British Cohort Study. *Am J Gastroenterol* 2000; **95**: 3507-3512 [PMID: 11151885]
81 **Feeney M**, Ciegg A, Winwood P, Snook J. A case-control study of measles vaccination and inflammatory bowel disease. The East Dorset Gastroenterology Group. *Lancet* 1997; **350**: 764-766 [PMID: 9297995]
82 **Nielsen LL**, Nielsen NM, Melbye M, Sodermann M, Jacobsen M, Aaby P. Exposure to measles in utero and Crohn's disease: Danish register study. *BMJ* 1998; **316**: 196-197 [PMID: 9468686]
83 **Jones P**, Fine P, Piracha S. Crohn's disease and measles. *Lancet* 1997; **349**: 473 [PMID: 9040582]
84 **Hansen TS**, Jess T, Vind I, Elkjaer M, Nielsen MF, Gamborg M, Munkholm P. Environmental factors in inflammatory bowel disease: a case-control study based on a Danish inception cohort. *J Crohns Colitis* 2011; **5**: 577-584 [PMID: 22115378 DOI: 10.1016/j.crohns.2011.05.010]
85 **Haslam N**, Mayberry JF, Hawthorne AB, Newcombe RG, Holmes GK, Probert CS. Measles, month of birth, and Crohn's disease. *Gut* 2000; **47**: 801-803 [PMID: 11076878]
86 **Ghosh S**, Armitage E, Wilson D, Minor PD, Afzal MA. Detection of persistent measles virus infection in Crohn's disease: current status of experimental work. *Gut* 2001; **48**: 748-752 [PMID: 11358885]
87 **Haga Y**, Funakoshi O, Kuroe K, Kanazawa K, Nakajima H, Saito H, Murata Y, Munakata A, Yoshida Y. Absence of measles viral genomic sequence in intestinal tissues from Crohn's disease by nested polymerase chain reaction. *Gut* 1996; **38**: 211-215 [PMID: 8801199]
88 **D'Souza Y**, Dionne S, Seidman EG, Bitton A, Ward BJ. No evidence of persisting measles virus in the intestinal tissues of patients with inflammatory bowel disease. *Gut* 2007; **56**: 886-888 [PMID: 17519494]
89 **Koutsoumpas AL**, Polymeros D, Tsiamoulos Z, Karamanolis G, Triantafyllou K, Bogdanos DP, Ladas SD. S1191 Molecular Mimicry and Immunological Crossreactivity Between Measles Virus and Human Intestinal Proteins is Not a Feature of Crohn's Disease. *Gastroenterol* 2010; **138** (suppl 1): S-200
90 **Davis RL**, Bohlke K. Measles vaccination and inflammatory bowel disease: controversy laid to rest? *Drug Saf* 2001; **24**: 939-946 [PMID: 11735650]
91 **Montgomery SM**, Morris DL, Pounder RE, Wakefield AJ. Paramyxovirus infections in childhood and subsequent inflammatory bowel disease. *Gastroenterology* 1999; **116**: 796-803 [PMID: 10092301]
92 **Ng SC**, Woodrow S, Patel N, Subhani J, Harbord M. Role of genetic and environmental factors in British twins with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 725-736 [PMID: 21557397 DOI: 10.1002/ibd.21747]
93 **Folwaczny C**, Jäger G, Schnettler D, Wiebecke B, Loeschke K. Search for mumps virus genome in intestinal biopsy specimens of patients with IBD. *Gastroenterology* 1999; **117**: 1253-1255 [PMID: 10610334]
94 **Iizuka M**, Saito H, Yukawa M, Itou H, Shirasaka T, Chiba M, Fukushima T, Watanabe S. No evidence of persistent mumps virus infection in inflammatory bowel disease. *Gut* 2001; **48**: 637-641 [PMID: 11302960]
95 **Waters O**, Ahmad T. Opportunistic infections and vaccinations in IBD patients. *J Crohns Colitis* 2011; **5**: 263; author reply 264 [PMID: 21575894]
96 **Sipponen T**, Turunen U, Lautenschlager I, Nieminen U, Arola J, Halme L. Human herpesvirus 6 and cytomegalovirus in ileocolonic mucosa in inflammatory bowel disease. *Scand J Gastroenterol* 2011; **46**: 1324-1333 [PMID: 21879802 DOI: 10.3109/00365521.2011.605466]
97 **Lidar M**, Langevitz P, Barzilai O, Ram M, Porat-Katz BS, Bizzaro N, Tonutti E, Maieron R, Chowers Y, Bar-Meir S, Shoenfeld Y. Infectious serologies and autoantibodies in inflammatory bowel disease: insinuations at a true pathogenic role. *Ann N Y Acad Sci* 2009; **1173**: 640-648 [PMID: 19758210 DOI: 10.1111/j.1749-6632.2009.04673]
98 **Yanai H**, Shimizu N, Nagasaki S, Mitani N, Okita K. Epstein-Barr virus infection of the colon with inflammatory bowel disease. *Am J*

Gastroenterol 1999; **94**: 1582-1586 [PMID: 10364028]
99 **Linton MS**, Kroeker K, Fedorak D, Dieleman L, Fedorak RN. Prevalence of Epstein-Barr Virus in a population of patients with inflammatory bowel disease: a prospective cohort study. *Aliment Pharmacol Ther* 2013; **38**: 1248-1254 [PMID: 24053733 DOI: 10.1111/apt.12503]
100 **Sankaran-Walters S**, Ransibrahmanakul K, Grishina I, Hung J, Martinez E, Prindiville T, Dandekar S. Epstein-Barr virus replication linked to B cell proliferation in inflamed areas of colonic mucosa of patients with inflammatory bowel disease. *J Clin Virol* 2011; **50**: 31-36 [PMID: 21035384 DOI: 10.1016/j.jcv.2010.09.011]
101 **Subramaniam K**, D'Rozario J, Pavli P. Lymphoma and other lymphoproliferative disorders in inflammatory bowel disease: a review. *J Gastroenterol Hepatol* 2013; **28**: 24-30 [PMID: 23094824 DOI: 10.1111/jgh.12015]

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