Pathogenesis of inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) constitutes two related clinical entities, Crohn’s disease (CD) and ulcerative colitis (UC), which resemble each other so closely that they cannot be distinguished even pathologically, but differ from each other sufficiently to regard them as independent entities. Both are caused by a disruptive interaction between the immune system and intestine luminal factors. The exact etiology of IBD remains unclear, but clinical and laboratory studies indicate that both genetic and environmental factors are important. Thus, it is of high importance to deepen our knowledge about the pathogenesis of IBD not only to make a quick diagnosis but also to investigate new methods of treatment.

Key words: inflammatory bowel disease, pathogenesis, inflammation, immune system

Introduction

Inflammatory bowel disease (IBD) constitutes two related clinical entities, Crohn’s disease (CD) and ulcerative colitis (UC), which resemble each other so closely that they cannot be distinguished even pathologically, but differ from each other sufficiently to regard them as independent entities. Both are caused by a disruptive interaction between the immune system and intestine luminal factors. The main differences however, is the localisation and nature of the inflammatory changes. CD can occur in any part of the digestive system, although a majority of the cases start in the terminal ileum. UC in contrast, is restricted to the colon and the rectum. Microscopically, UC is restricted to the mucosa while CD affects the whole bowel wall. They may present with any of the following symptoms: abdominal pain, vomiting, diarrhea, hematochezia (bright red blood in stools), weight loss as well as various associated complaints or diseases like arthritis, pyoderma gangrenosum, and primary sclerosing cholangitis. Still, they present with extra-intestinal manifestations, such as liver problems, arthritis, skin manifestations and eye problems in different proportions. Diagnosis is generally made by colonoscopy with biopsy of pathological lesions [5, 6, 59]. The exact etiology of IBD remains unclear, but epidemiological observations may be helpful in identifying its true causative factors. Numerous studies in different populations offer interesting data which reflect genetic, inherited, environmental and behavioural factors [4]. Recently published genome-wide association studies (GWAS) also highlight the importance of the immune system and its interactions with the intestinal flora in IBD pathogenesis [26].

Environmental factors

It is reported that incidence of IBD has been increasing world-wide of late, but its spread has been slowing down
in highly affected countries. Geographically, the prevalence of IBD has a slope from North to South and, to a lesser degree, from West to East. The Western-Eastern discrepancy can be attributed to a difference in Western life styles – there is no doubt that economic development, leading to improved hygiene and other changes may play a role in the increase in both CD and UC [26, 28]. Moreover, the disease seems to have a characteristic racial-ethnic distribution: the Jewish population is highly susceptible everywhere, when Gypsies have a considerably lower prevalence than the average population (specially in Hungary). Currently, behavioral influences are also investigated. Diet (e.g. refined sugar, fat, and fast food), the role of the early ages, smoking habits, hormonal status and drugs (specially nonsteroidal anti-inflammatory medicines) are viewed as useful contributing factors in the manifestation of IBD. According to age, the onset of the disease occurs more often in the second or the third decade of life, but there also is another peak in the 60s. Regarding sexual distribution, there is a slight preponderance of UC in men and of CD in women [1]. Smoking is the ambiguous factor. A meta-analysis partially confirms previous findings that nicotine was found to be protective UC and, after the onset of the disease, might improve its course, decreasing the need for colectomy. In contrast, smoking enlarge the risk of developing CD and aggravates its course by increasing the need for steroids, immunosuppressants and re-operations, but an active nicotineism does not affect the outcome while passive smoking is detrimental for the outcome of CD patients. Data are however, largely conflicting and the potential mechanisms involved in this dual relationship are still unknown [28, 29, 56]. Other factors such as oral contraceptive use, appendectomy, perinatal events, and childhood infections have also been associated with both diseases, but their role is more controversial [26].

Hypotheses regarding pathogenesis

Although many questions remain regarding the etiology of UC and CD, clinical and laboratory studies indicate that both genetic and environmental factors are important. Several theories about the pathogenesis of IBD have been entertained, including an autoimmune response to a luminal or mucosal antigen, a dysfunctional immune response to a commensal bacterium, and an infection with a pathogenic organism which remains in the intestinal tissues and results in a chronic inflammatory response. A variation of the last hypothesis involves persistent abnormalities in immune regulation following an acute infection with a pathogenic organism that is cleared.

Autoimmune reactions

The mucosal immune system of the intestine plays a central role in the pathogenesis of IBD. In the healthy gut, the mucosal immune system ensures the balance between pro- and anti-inflammatory mediators and thereby allows an effective defense against luminal pathogens but at the same time prevents an overwhelming immune reaction directed against the huge amount of harmless luminal antigens, e.g. components of food or nonpathological bacteria. In both entities of IBD this immunological balance is severely impaired and shifted towards the pro-inflammatory side [56]. Destructive inflammatory response directed toward a self-antigen such as mucin, goblet cells, colonocytes, or other cells has been proposed as the underlying basis of IBD, particularly in regards to UC [19, 27, 30, 36]. As noted above, antibodies against neutrophils have been found in many but not all UC patients [11, 30, 35]. However, the titers of antineutrophil antibodies do not seem to correlate with disease activity in UC, and their biological significance in UC patients is unclear [40]. Levels of antibodies against a human intestinal tropomyosin isoform also have been reported to be increased in UC patients in some studies [30, 36]. In addition, activated complement factors have been noted to colocalize with anticolon antibodies on the luminal surface of the epithelium [23]. Exposure to microbial peptides that share immunogenic determinants with self-antigens has been suggested as the trigger for the disruption in immune tolerance to endogenous gut antigens. For example, some studies have implied that antibodies or T cells recognizing mycobacterial heat shock protein HSP65 and human HSP60 may be involved [23]. However, other investigations have failed to confirm these findings.

Intestinal microbiota

Accumulating data, including GWAS highlight and further support the importance of the gut homeostasis and IBD pathogenesis. Although large numbers of bacteria colonize the lumen of the intestine, several studies have revealed that under normal conditions the intestinal mucosa is relatively free of adherent bacteria [2, 24, 43, 52]. Thus, IBD patients may have alterations in the mucus-epithelial layer which allow a more intimate association of gut bacteria with the mucosa. Potentially, the presence of mucosa-associated indigenous bacteria in these patients produces disease despite the absence of more aggressive pathogenic bacteria. The closer proximity of the bacteria to the epithelium may be a crucial component in the initiation of the cycles of inflammation and changes in intestinal permeability that are characteristic of IBD. Genetic factors may contribute to both the increased penetration of the bacteria or bacterial products as well as the pronounced inflammatory response that occurs. The clinical presentation and histologic appearance of IBD has a number of similarities to gastrointestinal infections by known pathogenic organisms. For instance, acutely CD can appear like gastrointestinal disease due to *Yersinia* or *Mycobacterium tuberculosis*. Infection with other enteric pathogens such as *Shigella* or *Campylobacter* can strongly resemble UC [23]. However, clinical and immunohistochemical studies have failed to provide convincing evidence of a role for mycobacteria in CD [23, 47, 58]. In recent years, a number of studies utilizing polymerase chain reaction (PCR) methods have addressed the mycobacterial hypothesis. The majority of these investigations used primers recognizing IS900, a multicopy genomic DNA insertion element highly specific for *Mycobacterium paratuercu*
nosis. Most of the PCR studies suggest that mycobacterial sequences can be detected in intestinal tissue but that their presence is not specific for CD [23, 58]. Many efforts were also made to assess the role of gut microbiota in different age groups. The observations revealed an increase in anaerobic bacteria (Bacteroides vulgatus, Streptococcus faecalis) in adult IBD, whereas an increase in aerobic and facultative-anaerobic (Escherichia coli) in pediatric IBD. Overall higher bacterial cell counts were observed in IBD, jointly with a general loss of biodiversity and a preponderance of Bacteroidetes and a parallel decrease of Firmicutes, particularly the species Firmicutes praunisztii. A predominance of potential harmful members of Proteobacteria (Escherichia coli) and low abundance of beneficial species (Faecalibacterium prausnitzii) was also reported in pediatric and adult age groups, respectively. This breakdown in the balance between putative species of ‘protective’ versus ‘harmful’ intestinal bacteria been termed dysbiosis resulting in decreased bacterial diversity [10].

Wakefield et al. have suggested that persistent measles infection, particularly when exposure occurs in utero or early in life, may lead to CD [51, 53]. This hypothesis proposes that CD is a chronic granulomatous vasculitis in reaction to a persistent infection with measles virus, which exhibits a tropism for the submucosal endothelium of the intestine. Epidemiologic evidence from Sweden has suggested a link between CD and early measles infection [12, 13]. In addition, paramyxovirus-like structures have been visualized in the vascular endothelium of CD patients by electron microscopy and measles antigen has been detected in areas of granulomatous inflammation [51, 53]. However, other investigators have not been able to confirm an epidemiological link between CD and early measles infection [3, 15, 22] or to confirm the association by immunohistochemical staining or serologic studies [18, 23, 25].

Nevertheless, in IBD patients, increased levels of mucosal antibodies directed against different intestinal bacteria have been observed [42, 46]. In addition, the clinical response of CD patients to antibiotics and the beneficial effect of fecal diversion in selected CD patients with perianal disease suggest an involvement of bacteria in the disease symptoms [8, 49, 60].

**Inflammatory mediators**

Number of studies have suggested that CD is a Th1-mediated disease and that excessive Th1-cell activity is a critical component of CD [30, 37]. Interleukin (IL)-12, IL-18 which are involved in Th1-cell development and interferon (IFN)-γ production, were found at increased levels in the intestinal mucosa of patients with CD [32, 38]. Tumor necrosis factor (TNF) is also an important mediator of the intestinal inflammation in CD. Several clinical trials have shown a chimeric anti-TNF-α antibody to be effective in the treatment of CD, verifying the importance of TNF in the ongoing intestinal inflammation [39, 41, 45, 57]. Increased levels of IL-15 production by CD lamina propria T lymphocytes also have been reported [8]. IL-15 is a cytokine with a number of biological activities including the stimulation of T-cell proliferation and migration to sites of inflammation. Recently, transcription nuclear factor (NF)-kappaB was identified as one of the key regulators in this immunological setting. Its activation is markedly induced in CD patients and through its ability to promote the expression of various pro-inflammatory genes, it strongly influences the course of mucosal inflammation. Pharmacological attempts to block the activation of NF-kappaB develop new therapeutic strategies in CD [34]. Metallothioneins (MTs) that are a family of small proteins with a high and conserved cysteine content are rapidly upregulated in response to an inflammatory stimulus. It exert a central position in zinc homeostasis, modulate the activation of NF-kappaB, and serve as antioxidants. They are probably involved in CD through their antiapoptotic effects or through specific immunomodulating extracellular effects [50].

Less information is available regarding the pathogenesis of UC. However, several studies suggest that UC differs from CD in the profile of cytokines in the intestinal mucosa [37]. For example, Th2 cells rather than Th1 cells have been hypothesized to play a prominent role in UC, but only limited data are available to confirm this idea. In support of this notion, though, IL-12 transcripts have been found in the intestinal mucosa of CD patients but not generally in UC [28, 68]. Also, the UC intestine has many immunoglobulin G (IgG)-secreting plasma cells and IgG1 colocalizing with the complement component C3b on the surface of epithelial cells [30]. In addition, in UC evidence for an abnormal humoral immune response exists, such as the presence of antibodies to the perinuclear component of neutrophils as mentioned above. Nonetheless, although lamina propria T cells from UC patients have been reported to secrete greater amounts of IL-5 than do those from controls, the production of IL-4 does not appear to be increased in UC, which would be expected with a Th2-cell response [20, 30, 37].

**Genetic factors**

It has long been recognized from family and twin studies that IBD, have a strong genetic predisposition, interacting with unknown environmental drivers to render susceptible individuals at risk for relapsing intestinal inflammation. Substantial progress has been made in the last 2 years in characterizing the susceptibility genes involved. GWAS have robustly identified 11 susceptibility genes and loci and highlighted a number of new, previously unsuspected pathways as playing an important role in IBD pathogenesis. The most pathophysiologically relevant associations reported so far are with gene variants related to innate immunity, autophagy, apoptosis, Th1 and Th17 responses, T cell activation (CTLA-4 Gene Promoter [49 A/G] in exon 1 polymorphisms), and immunosuppression. The most consistently and strongly associated variants have been in the IL23 receptor – an uncommon coding variant rs11209026 (protection against IBD overall ), the caspase recruitment domain family member 15 (CARD15/Nod2) and autophagy-related 16-like 1 (ATG16L1-rs2241880) genes (risk only for CD) [1, 17, 21, 55, 61]. Quite recently, UC-associated variants
have also emerged. There is growing evidence that proteins involved in the apical junction complex (PTPRS) as well as a variant in ABCB1 (ATP-binding cassette subfamily B member 1) are involved in this disease. Additionally, new collaborative studies proved that genetic variation in myosin IXB (MYO9B) – the cytoskeletal protein playing role in epithelial cell junctions, is also significantly associated with UC while weakly with CD [9, 16, 55].

One of the first single nucleotide polymorphisms (SNPs) that was investigated to evaluate the role of genetic factors in the pathogenesis of IBD was NOD2/CARD15 (R702W, G908R and L1007fsnc). As yet, it is only partially understood how mutations in this particular gene lead to CD. Mouse models, in vitro data and studies in humans offer conflicting data as regards whether there is a loss or gain of function of NOD2 in bowel inflammations. Till now, several additional genes such as TLR4, MDR1, NOD1 (CARD4), DLG5 as well as the IBD5 locus including SLC22A4/S5 have been identified as potential disease causing or disease modifying genes [54]. Recently, the evidence for genetic interactions between polymorphisms in TLR9 and CD-associated variants in NOD2, IL23R, and DLG5, differentially modulating CD susceptibility has been provided [48]. With respect to ATG16L1, the G allele of SNP rs2241880 has been shown in multiple association studies to confer strong risk for CD with specific focus on how its protein product operating within the autophagic pathway makes autophagy an attractive therapeutic target for this debilitating disorder [21].

Neuropeptides

It has become increasingly evident that interactions between the enteric nervous system and the immune system play an important role in the cause of IBD. Both the enteric nervous system (ENS) and the central nervous system (CNS) can amplify or modulate the aspects of intestinal inflammation through secretion of neuropeptides (substance P, corticotropin-releasing hormone, neurotensin, and vasoactive intestinal peptide) or small molecules (acetylcholine and serotonin) [31]. It is not known to date whether these changes in neuropeptides are due to altered synthesis and release from intrinsic and/or extrinsic neurons and nerve fibers. The changes in circular smooth muscle response associated with diminished VIP in the intestine of CD patients suggests that VIP may play an important role in the pathophysiology of motility in IBD [14]. The pronounced increase in SP receptors at small vessels in all gut layers and at lymph nodules in the inflamed intestine of IBD patients supports the hypothesis that SP is a modulator of inflammation in IBD and possibly acts by release from extrinsic sensory nerves of the gut. Sensory nerve may play a role not only in enhancing an inflammatory response in the intestine, but also in tissue repair. An inflammatory response after tissue injury and subsequent wound healing presumably is the normal response in healthy tissue. In IBD however, this sequence may be deeply disturbed by an unrestricted immune response which does not lead to or delays intestinal tissue healing. Although it is intriguing to postulate that interactions between the immune system and nervous system exist and play a role in the pathophysiology of intestinal inflammation, in vivo studies blocking or mimicking neuropeptide action are needed to prove this bidirectional communication. Unfortunately the complexity of neuroimmune-endocrine systems, conflicting study results and dual mechanisms of action, warrant further research in this field. Clarification of the molecular mechanisms of action of neuropeptides and on immune and inflammatory reactions will likely yield new treatment options in the future.

Cholesterol oxidation products

Cholesterol oxidation products (Oxysterols), both of dietary and endogenous origin, are suspected to have pro-oxidant and pro-apoptotic effects on human colonic epithelial and thus are considered to be potentially involved in the initiation and progression of IBD [7, 44]. They affect only differentiated Caco-2 colonic cells, mainly because of their very low AKT phosphorylation pathway as to the undifferentiated counterpart. Oxysterols of pathophysiologic relevance generally possess a strong pro-oxidant effect, chiefly since they activate NAD(P)H oxidases. Further, stimulation of the MEK/ERK signaling pathway appears to be a common feature of the biochemical effects of this class of compounds. These findings point to the novel therapeutic targets such as statin-based therapy. Selective metabolic inhibitors of NAD(P)H oxidase and the MAPK pathway might quench or even prevent the cytotoxic effects of pathological accumulation of cholesterol oxides in cells and tissues. The marked reduction of plasma oxysterols might thus be considered as prevention not only of IBD but generally of human “multifactorial” disease processes.

Summary

The exact etiology of IBD still remains unclear although numerous studies are performed on this subject. Different data reflect genetic, inherited, environmental and behavioral factors as its causative agents. Recent investigations also highlight the importance of the immune system and its interactions with the intestinal flora. Inherited predispositions however, seems to be the most important and best investigated factor. The immense evolutions in genetic researches provide contributions in the development of new therapeutic approaches. Although, a new challenges are still appearing, great and significant progress has already been done.

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References


