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VIEWPOINT

Why does Crohn's disease usually occur in terminal ileum?

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Abstract

Crohn's disease can affect any part of the gastrointestinal tract, but terminal ileum is the most frequent localization. The reason why Crohn's disease is primarily located in the distal part of the ileum remains unexplained.
In this article it has been attempted to provide a compelling explanation why Crohn's disease usually occurs in terminal ileum. Recent data indicate that some individuals are genetically predisposed to develop ileal Crohn's disease. Two genetic alterations, the polymorphism of Caspase Associated Recruitment Domain (CARD15) and Carcinoembryonic Antigen-related Cell Adhesion Molecule 6 (CEACM6), favour the colonization of terminal ileum by entero adherent-invasive *Escherichia coli* (AIEC). The adhesion of these bacteria to epithelial intestinal cells depends on Carcinoembryonic Antigen-related Cell Adhesion Molecule 6 expression in ileal epithelial cells and on the reduced ileal defensins expressed in a CARD15 dependent manner. Genetic defects in Authophagy-related 16-like gene (ATG16L1) and Immunity-related Guanosine Triphospatase (IRGM) recently found in ileal CD patients lead to a reduction of bacterial killing by macrophages and consequent continuous immunological upstimulation, cytokine secretion, chronic inflammation of the ileum and tissue injury. On the basis of all these data Crohn's disease of the ileum seems to be a subset of the disease mainly genetically determined.

1. Introduction

Crohn's disease (CD) can affect any part of the gastrointestinal tract but terminal ileum is the most frequent localization as recognized by B. Crohn in its original description.[1] According to Vienna Classification that subdivides the site of disease in terminal ileum, colon and ileum and colon, the ileal localization occurs in two thirds (57–89%) of the patients and isolated ileal disease is present in at least one third.[2] It is also well known that the most common localization of postoperative recurrence after curative ileocolonic resection for CD is the neoterminal ileum.[3]

Despite the tremendous progress in our understanding of the pathogenesis of inflammatory bowel diseases (IBD) the reason why CD is primarily located in the distal part of the ileum remains unexplained. However, in the last few years a constellation of findings mainly genetic in nature have given a great contribution to the genotype–phenotype correlations.

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Taken together these arguments seem to provide a rational hypothesis to explain the enigma of terminal ileitis. The main arguments in favour of the distal ileal localization of CD will be discussed down here.

2. Peyer's patches distribution

The bowel is a key component of the immune system, represents the most extensive surface exposed every day to a massive antigenic load and is able to distinguish between invasive pathogens and innocuous antigens from food and commensal bacteria. When CD develops tolerance to innocuous antigens is lost. Defensive mechanisms are regulated by gut-associated lymphoid tissue (GALT) represented by Peyer's patches, mesenteric lymphonodes, cryptopatches and isolate lymphoid follicles/lymphocyte filled villi.[4] Peyer's patches are the most populated structures by immune cells and are located only in the small bowel and particularly in the distal ileum. The predominant localization in the distal ileum of the Peyer's patches, which are the most complex part of GALT, fit with the most frequent localization of CD to distal ileum. It has also been shown that the great peak of incidence of CD occurred at ages 15 to 25 years and paralleled a similar peak representing the number of Peyer's patches as a function of age.[5] In addition CARD 15 deficiency induces an abnormal development of Peyer's patches characterized by an exaggerated immune response and an increased permeability.[6]

3. NOD2/CARD15 mutations

Many studies have identified putative loci on several chromosomes to localize Inflammatory Bowel Disease (IBD) susceptibility genes through genome-wide linkage studies.[7] In 1996, Jean-Pierre Hugot's group identified a susceptibility locus for CD adjacent to the centromere on chromosome 16 (IBD1), confirmed by a number of centres (notably by the IBD International Genetics Consortium).[8] In 2001, three independent mutations within NOD2 gene (renamed CARD15 by the International Nomenclature Committee) mapping to chromosome 16, were found to be associated with CD.[9–11] The identified mutations included one frameshift mutation (3020insC) and two missense mutations (C2104T and C2722C).

In several studies, a significant association was found between ileal disease and the carriers of one or more CARD 15 risk variant alleles, particularly with double-dose carriers.[12–14] The CD patient with ileal disease has a 2.5-fold risk of being a carrier of at least one of three CARD15 mutations[15]. The association of CARD15 mutations with ileal disease suggests the existence of an important difference between the immune tolerance mechanisms of the ileum and the colon. Indeed, the colon, unlike ileal mechanisms, may make use of immune mechanisms that do not depend on intact CARD15 functions. In an Italian population we have confirmed that CARD15 mutations are significantly associated with small bowel location and with acute intestinal obstruction at diagnosis.[16]

CARD15 is an intracellular receptor protein involved in the recognition of intracellular pathogen-associated molecular patterns such as lipopolysaccharide (LPS), peptidoglycan (PGN) and/or invasive bacteria.[17] This receptor plays a key role in detecting the bacterial antigen and in transmitting several signaling cascades that induce a complex innate gene programme.[18] Its structural domains are related to the well-described R proteins in plants that mediate host resistance to microbial pathogens. There are three structural domain of CARD15 protein: the N-terminus portion containing two space-associated recruitment domain (CARD) which induce the nuclear factor-kB (NfkB) signaling cascade; the central nucleotide-binding domain which induces self-oligomerisation required for activation; and the C-terminus leucine-rich appear (LRR) domain which is a pattern-recognition receptor for several types of microbial components.[18] The three CARD15 mutations have been identified within or near the LRR domain.

CARD15 was first demonstrated to be expressed by monocyte/macrophage cells,[17] but recent studies provided important new information concerning CARD15 function and regulation also in intestinal epithelial cells (IECs), which are a primary element of the intestinal barrier.[19–22] These studies demonstrated that CARD15 is expressed both in epithelial cell lines and primary IECs. Indeed, in CD patients, CARD15 is up-regulated in colonic epithelial cell compared to normal controls. The tumour necrosis factor-α (TNF-α) with interferon-γ (IFN-γ) up-regulate CARD15 protein and Mrna levels in IECs through an NFkB-dependent mechanism.[20] Moreover, CARD15 is expressed and functions as a defensive factor against intracellular bacteria in IECs, initiating a gene programme aimed at eliminating invasive intracellular bacteria. In fact, it has recently been reported that CARD15 protein appears critical for eradicating intracellular Salmonella typhimurium in IECs.[21] The frameshift mutation 3020InsC produces a truncated CARD15 protein incapable of sensing LPS and of initiating NFkB signaling and impairs its ability to eliminate invasive bacteria in IECs. The bacterial clearance was accelerated in cells expressing a functional CARD15 protein, whereas cells expressing the 3020InsC mutant were unable to clear the pathogen.

It is of note that the CARD15 receptor protein is the functionally intracellular counterpart of the cell surface Toll-like receptors (TLRs).[23] This family of proteins is composed of 10 types of receptors, transducing specific microbial antigens. They play a pivotal role in microbial recognition, induction of antimicrobial genes and control of adaptive immune responses.[24] TLRs are widely expressed on several types of cells present in the gastrointestinal mucosa and maintain homeostasis between high antigen load (more than 10^{11} microorganisms per gram of luminal contents can be found in the colon), immune and non-immune cells of the gastrointestinal mucosa.[25] Disruption of this delicate balance could, potentially, lead to chronic intestinal inflammation, as in CD, where dysregulated and/or constant activation of the innate system has been clearly shown.[26]

4. Defensins defect

Recent observations showed a link between CARD15 mutations and defensin expression in CD.[27]

Defensins are small peptides with a molecular weight of 3–5 KDa and are classified as α-and β-defensins. α-defensins include human defensin 5 (HD-5) and 6 (HD-6).

Human α-defensins are antibiotic effector molecules, that rapidly kill a broad range of microorganism,[28] predominantly expressed in Paneth cells of the ileum. Since Paneth cells also

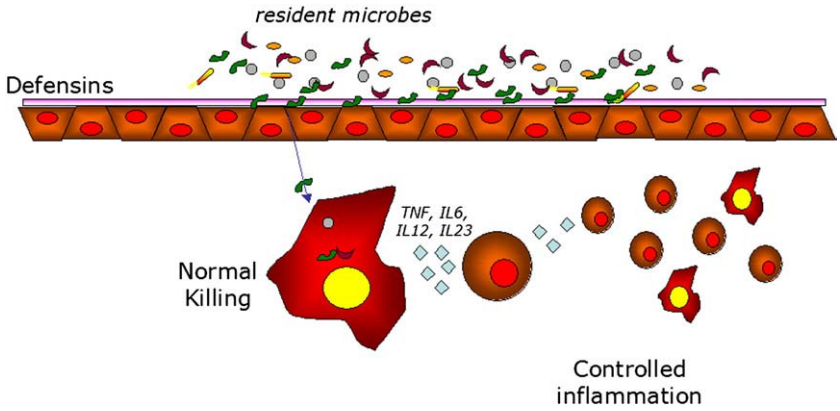

Figure 1 Terminal ileum: homeostasis. Dynamic balance between resident microbes and host defensive response. In normal subjects the Paneth cells of terminal ileum express defensins that kill a broad range of microorganisms of luminal flora defending the integrity of mucosal barrier. Commensal bacteria activate a sequential program of homeostatic responses by epithelial cells, macrophages, dentritic cells and T lymphocytes that lead to mild accumulation of lymphocytes and other cells in the lamina propria (controlled inflammation).

express CARD15,[29] it has been postulated that CARD15 may regulate Paneth cell defensin expression or affect the number of Paneth cells.[27] Ileal expression of human defensins (HD_5, HD_6) were found to be diminished in CD affected ileum and the decrease was more pronounced in patients with CARD15 mutations.[29]

The reduction of ileal defensin production in CD patients with CARD15 genotype is another argument fitting with the preferred ileal localization of the disease.

5. Bacteria adherence/invasion

CD is a multifactorial disease but is generally accepted that luminal bacteria play an essential role in the pathogenesis. The luminal bacteria induce physiologic and pathophysiologic important immune responses and are essential for the development of intestinal inflammation.

The distal ileum has a very high luminal concentration of predominantly anaerobic bacterial and fungi (10^{-7}–10^{-8}).[30] It has been shown that IBD patients have a decrease in the number of Bifidobacterium and Lactobacillus spp. and an increase in pathogenic bacteria such as Bacterioides and *Escherichia coli* (*E. coli*).[31] Accumulate evidence indicates that the balance between commensal flora and host defensive responses at the mucosal level has a pivotal role in the initiation of IBD. Although a number of microorganisms have been implicated in CD pathogenesis, *E. coli* is presently the most actively investigated.

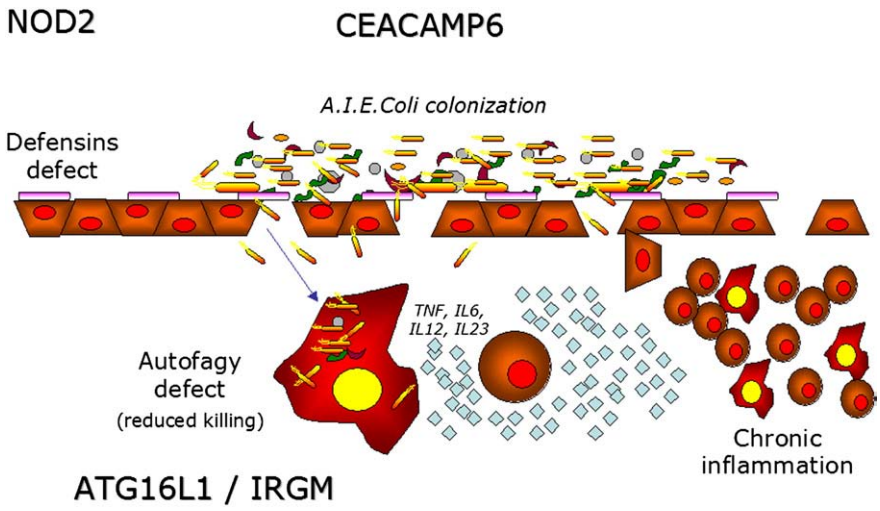

Figure 2 Pathogenesis of Crohn's disease of the ileum. Epithelial attachment, invasion and tissue injury by Adhesive/invasive *E. coli* (AIEC) in ileal CD. In patients with CARD15 mutations ileal defensins are diminished with consequent colonization and translocation of intestinal bacteria. Abnormal carcinoembryonic antigen related cell adhesion molecular 6 (CEACAM 6) expression acts as receptor for AIEC and is another factor responsible for selective colonization and invasion of the ileal mucosa by AIEC. Bacterial strains interact with macrophages of the mucosa. An autophagy defect secondary to ATG16LI and IRGM gene mutations is responsible for a defective mucosal killing, resulting ineffective clearance of bacterial antigens, progressive hyperstimulation of immune response and chronic inflammation (uncontrolled inflammation).

E. coli is the predominant facultative aerobe of the human intestinal flora. Some species of *E. coli* are pathogenic due to several virulence factors (enteroinvasive, enterotoxigenic, enteropathogenic, enterohemorragic, enteroaggregative, enteroadhering).The enteric flora of IBD patients includes, more commonly than controls, strains of adherent/invasive Coli (AIEC).[32,33] The high levels of AIEC colonizing the intestinal mucosa of patients with CD strongly suggest that it plays an important role in the etiopathogenesis of CD. These *E. coli* strains are able to survive within macrophages and to induce the secretion of TNFα.[32] It has been recently shown that CD-associated AIEC strains adhere to the brush border of ileal enterocytes isolated from CD patients but not controls without IBD.[33] The same authors have demonstrated that carcino embryonic antigen related cell adhesion molecule 6 (CEACAM6) act as a receptor for AIEC, supporting the concept of ileal mucosa concentration in CD.[34] AIEC adhesion is dependent on type -1 pilus variant expression on the bacterial surface.[35] Commensal *E. coli* strains are able to adhere to enterocytes from CD patients but not from normal subjects. It appears therefore that in addition to the expression of CARD15 variant, abnormal ileal CEACAM6 expression is another factor responsible for abnormal colonization and invasion of the ileal mucosa by AIEC. Adhesion enables the bacteria to create a biofilm on the mucosa surface and to resist mechanical removal from the intestine. Due to their invasivity AIEC strains can interact with resident macrophages and activate immune cells. While most of invasive bacteria induce death of infected macrophages, no necrosis no apopthosis is observed after infection with AIEC.[33] It appears therefore that CD patients have a defective mucosal killing resulting in increased exposure to commensal bacteria and activation of T cells.

It is of note that AIEC was recovered from 100% of the biopsies of early lesions in postoperative endoscopic recurrence in CD[32] and that high counts of *E. coli* are associated with early recurrence.[36] These observations clearly support the classical experiment of Rutgeerts on the effect of fecal stream diversion on postoperative recurrence of CD in the neo-terminal ileum. The pathogenetic role of luminal content was suggested by prevention of postoperative recurrence by diversion of the fecal stream and appearance of inflammation soon after the reinfusion of ileostomy content.[37]

6. Autophagy and defective microbial killing

Recently a defect in autophagy has been identified in predisposing to CD. Autophagy is a catabolic process involving the degradation of a cell's own components through the lysosomal machinery. Autophagy plays an important role in the destruction of some bacteria within the cells and in the clearance of apoptotic bodies.[38] Alterations in autophagy leading to a defective intracellular responses to low-level invasive bacteria have been recently suggested in the pathogenesis of IBD, in particular CD.[39]

For many years it has been postulated that commensal intestinal bacteria predispose to CD but without any evidence as to how they might do so. Recent studies showed that defects in autophagy might be the key part of the pathogenic pathway resulting in defective microbial killing, increased exposure to commensal bacteria and activation of T cells. A genome-wide association study has shown an association of CD with the autophagy related 16-like 1 gene (ATG16L1).[40] In particular it has been found an association of a threonine-to-alanine substitution (T300A) in ATG16L1 with CD. The 300 A/A genotype conferred a 1.65 fold risk of CD, with a 2.2-fold risk of ileal disease indicating that T300A variant was associated specifically with the ileal form of CD. The ATG16L1 risk genotype showed also a modest but significant association with ulcerative colitis.[41] The autophagy gene influences susceptibility and disease location but not childhood-onset.[42]

Another autophagy gene, the IRGM located on chromosome 5q33, has been found to be significantly associated with CD.[43] IRGM encodes another key component of autophagy and is involved in the elimination of intracellular bacteria very efficiently.[44]

These recent discoveries strongly indicate that defects of autophagy associated with selected decreased α-defensin production predispose to CD, and in particular to ileal CD.

7. The hypothesis

In summary, although many advances have been made in the understanding of CD pathogenesis, we still do not fully understand why CD is preferably localized to terminal ileum.

Recently it has been found that some individuals are genetically predisposed to develop ileal CD. Two genetic alterations, the polymorphisms of CARD15 and CEACM6, favour the colonization of terminal ileum by entero-adhesive *E. coli*. In fact AIEC adhesion to epithelial intestinal cells depends on CEACAM 6 expression on the apical surface of ileal epithelial cells and on the reduced ileal defensin expression in the presence of CARD15 mutations. Genetic defects in ATG16L1 and IRGM autophagy genes lead to a reduction of bacterial killing by macrophages with intracellular bacterial persistence, and consequent continuous immunological upstimulation, cytokine secretion (TNFα, IL6, IL12, IL23, ecc), chronic inflammation of the ileum and tissue injury (Figs. 1 and 2).

The practical implication of this hypothesis is that antibiotic or probiotic treatments should play a primary role in the early treatment of CD ileitis.

In conclusion, a constellation of genetic and environment data seems to converge on providing a compelling explanation why CD usually occurs in terminal ileum. These data may not yet give all the answers to the puzzle but they may indicate which questions need to be answered to solve the main enigma of IBD.

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