Defensin deficiency, intestinal microbes, and the clinical phenotypes of Crohn’s disease

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Abstract: Crohn’s disease is a chronic, inflammatory disease of the intestinal mucosa. Although intestinal bacteria are implicated in disease pathogenesis, the etiology is still unclear. The main location of disease is the small intestine (ileum) and the colon. Ileal disease has been linked to a mutation in the NOD2 gene. Defensins are antimicrobial peptides and are mainly expressed in Paneth cells, epithelial cells that also express NOD2. In the colon, defensins are expressed by enterocytes or metaplastic Paneth cells. Crohn’s disease patients with ileal involvement, compared with controls or Crohn’s patients without ileal involvement, have diminished expression of ileal Paneth cell defensins. This decrease is even more pronounced in Crohn’s patients displaying a NOD2 mutation. In contrast, Crohn’s disease of the colon is characterized by an impaired induction of β-defensins in enterocytes. The colonic expression of the constitutive β-defensin 1 is also decreased in the inflamed colonic mucosa, but this decrease is less specific to Crohn’s disease, as it can also be found in ulcerative colitis patients. In conclusion, the regional localizations of Crohn’s disease, ileal or colonic disease, can be linked to different defensin profiles. Crohn’s disease of the ileum is associated with diminished defensin expression in Paneth cells. Crohn’s disease of the colon is associated with diminished β-defensin expression in enterocytes. Thus, it can be speculated that decreased defensin levels lead to a weakened intestinal barrier function to intestinal microbes and might be crucial in the pathophysiology of Crohn’s disease. J. Leukoc. Biol. 77: 460–465; 2005.

Key Words: NOD2 · Paneth cells · probiotic bacteria · E. coli Nissle 1917 · Crohn’s disease

INTRODUCTION

Crohn’s disease is a chronic disease of the intestine characterized by a transmural inflammation of the gut. Although the distal ileum is affected in ~70% of patients, the disease can be located anywhere from the oral cavity to the rectum. By comparison, ulcerative colitis, which is also a chronic disease of the intestine, is restricted to the colon, and the inflammation is limited to the mucosa. Both diseases constitute the two major chronic inflammatory bowel diseases (IBDs) affecting one in 500 individuals. The principle treatment for both diseases is a suppression of the inflammatory process. Because of substantial side-effects and uncontrolled relapses, this therapy remains far from satisfactory for patients and physicians.

The etiology of Crohn’s disease and ulcerative colitis is still enigmatic. There is convincing evidence that the development of Crohn’s disease is associated with good hygiene standards. In developing countries, infectious intestinal diseases are extremely common, and yet, idiopathic IBDs, especially Crohn’s disease, practically do not exist. We have recently discussed the idea of an association of Crohn’s disease with the more “pathogen-free” living conditions in the Western world [1]. The first occurrence of Crohn’s disease often starts after a bacterial infection [2]. The classical interpretation of this infection-triggered beginning of this chronic inflammatory disease is a loss of mucosal tolerance toward the bacteria responsible for the inflammatory process. According to our hypothesis, the host with IBD may be more likely to contract an intestinal infection because of a defective mucosal barrier. These epidemiological factors have to be seen with the understanding of a certain genetic background, rendering the host susceptible to infections.

The recent finding that approximately one-third of Crohn’s disease patients have a loss of function mutation in the NOD2 gene represents a major advance [3–6]. NOD2 is a general sensor of peptidoglycan through muramyl dipeptide detection [7]. Initially fitting well with the more common understanding of the disease, the pathophysiology of NOD2 in Crohn’s disease was proposed to link to immunological dysregulation in monocytes [8]. Alternatively, intestinal epithelial cells and Paneth cells [9–11], which have also been demonstrated to express NOD2, might be compromised in their antibacterial response. It has been shown recently that intestinal epithelial cells transfected with mutated NOD2 are not able to respond appropriately to an in vitro challenge with Salmonella [12]. It is interesting that a mutation in the NOD2 gene, especially SNP13, is associated with ileal localization of disease [13]. As compared with monocytes, which are widely distributed, Pan-
eth cells and their main effector molecules [human defensin 5 (HD5) and HD6] are normally restricted to the small intestine, which fits the phenotype of ileal affection in Crohn’s disease. We and a growing number of others favor the hypothesis of a primary defect in the defensin-mediated antibacterial mucosal barrier, which is outlined and updated [1, 14] in this article. A simplified illustration of this concept is shown in Figure 1. A new and fascinating aspect of the recent data about defensin profiles in Crohn’s disease is the association of diminished defensin expression or lack of defensin induction to the main clinical phenotypes, ileal and colonic Crohn’s disease. As discussed in this article, ileal disease is associated with a diminished expression of the main ileal antibiotic effector molecules (HD5 and HD6). Conversely, Crohn’s colitis is associated with a lack of β-defensin induction. In this review, we will discuss known and recent findings, which are consistent with our general hypotheses of Crohn’s disease as a defensin deficiency syndrome [1, 14].

**PATHOPHYSIOLOGY: THE ROLE OF LUMINAL AND MUCOSAL BACTERIA**

For many years, there has been an ongoing discussion that IBDs are caused by a specific, hitherto unrecognized infection. The potential role of several pathogenic microbes, such as *mycobacterium*, *listeria*, and *rubella* (measle) infection and *saccharomyces*, has been discussed recently [1]. The multitude of identified mucosal pathogens or antibodies does not seem to be restricted to a single microorganism and suggests that the mucosa in Crohn’s disease is more susceptible in general to harbor commensal as well as potentially pathogenic bacteria. It is expected that research has focused initially on pathogenic bacteria in Crohn’s disease, but what about nonpathogenic bacteria, which are part of the bacterial flora? Does the flora change in Crohn’s disease? Does the ileum as the main location of the disease, which usually does not harbor many bacteria, or the colon mucosa contain more bacteria in Crohn’s disease?

First, there is a tremendous increase in the mucosal-associated bacterial counts in the neoterminal ileum after ileocolic resection for Crohn’s disease, and this colonization may be related to postoperative relapse [16]. As discussed recently [1], different groups, mainly from France and Germany, have demonstrated convincingly that the mucosa in IBD is characterized by adherent and sometimes invading *Escherichia coli* strains from the lumen [17, 18]. In contrast, normal mucosa is virtually sterile when washed a few times in saline. Although the causal link has not yet been proven, a breakdown in the expression or function of the antimicrobial mucosal barrier may well explain these findings, which have been reviewed recently by Linskens et al. [19]. An interesting study from the group in France, Darfeuille-Michaud and colleagues [20], described adherent, invasive *E. coli* strains specifically in the ileal mucosa of Crohn’s disease patients but not in ulcerative colitis. Another recent study has found increased levels of adherent *E. coli* in Crohn’s disease colonic mucosa as well as in colon cancer [21]. All of these findings fit well with the hypothesis of a break-in mucosal tolerance toward various luminal bacteria in IBDs [22]. To summarize, these findings indicate that Crohn’s mucosa may often be the target of various infections, but the proof that the disease is caused by these agents is missing. In addition, the immune response in the gut mucosa is not specific for any of these suspicious agents but rather, general response to a multitude of organisms. Rather, it is conceivable that there may be a primary defect in the peptide barrier of intestinal antibiotic defensins, which protect the normal mucosa extremely efficiently against adherent or invasive microbes. Rather than a specific infection, the commensal flora itself may cause inflammation in the absence of an adequate epithelial barrier function. Thus, not infection but rather the imbalance between the commensal flora and the host’s epithelium may be crucial to Crohn’s disease pathogenesis. We believe that a thorough understanding of these functionally relevant peptides is crucial for the understanding of mucosal biology, and an impaired balance of these effector molecules might explain many aspects of pathogenesis in IBDs.

**DEFENSIN EXPRESSION AND REGULATION IN THE INTESTINAL TRACT**

Many important studies in the field of defensins have focused on the human and nonvertebrate skin as another barrier of the body exposed to a multitude of bacteria. These studies resulted in the isolation of various peptides exhibiting potent antibiotic activity toward gram-positive and -negative bacteria, as well as enveloped viruses and fungi [23–25]. A similar system of antibiotic peptides is apparently synthesized and secreted by the intestinal mucosa as part of innate immunity but has received little attention, at least in the field of clinical gastroenterology. With more knowledge about defensins in the gas-

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**Fig. 1.** Simplified model of the normal reaction (upper) in the healthy intestine (modified figure from Huttner and Bevins [15]), as well as the defective antimicrobial barrier in the intestine of Crohn’s disease patients. In the healthy gut, the microbes cannot invade the mucosa because of an effective antimicrobial barrier (defensins). In Crohn’s disease (lower), especially in patients with ileal affection, this antimicrobial barrier is disturbed, and bacteria can invade the mucosa. According to this hypothesis, a bacterial invasion as a result of a defensin deficiency is the primary reason for the secondary inflammation.
trointestinal tract, especially over the past 5 years, we also begin to better appreciate the enormous complexity of expression and regulation of these peptides. Six α-defensins and four epithelial β-defensins have been identified in the different parts of human intestinal mucosa so far. The α-defensins comprise of human neutrophil peptides 1–4, produced by granulocytes, and HD5 and HD6, synthesized in Paneth cells [26].

REGULATION OF β-DEFENSINS

The β-defensins are of epithelial origin and abundant in skin, urogenital tract, intestine, and lung [27]. Defensins can be divided into constitutive forms, e.g., human β-defensin 1 (HBD-1) with its widespread, stable distribution [28], and inducible peptides such as HBD-2 [23]. The mechanisms of activation are still under investigation and are complex. Induction by cytokines, such as interleukin (IL)-1β and tumor necrosis factor α (TNF-α), has been shown in addition to a direct response to bacterial components, such as lipopolysaccharides (LPS) and lipoproteins [29, 30]. Possible signaling pathways involve Toll like receptors (TLRs), especially TLR2 and TLR4 [31, 32], but more work has to be done in various intestinal epithelial cells, which express various pattern recognition receptors recognizing microbial “pathogen-associated molecular patterns” as “nonself” to rapidly initiate innate immune responses of survival and to activate defense strategies against luminal pathogens [33, 34]. This system of several functional TLRs appears to be a key regulator of the innate response system. Different TLRs are responding to different pathogens and bacterial components, including among others, LPS and flagellin. In the context of pathogen recognition receptors, NOD2/CARD15 is an intracellular receptor for a peptidoglycan that induces nuclear factor (NF)-κB [35], which in turn is known to trigger HBD-2 transcription. In colonic epithelial cells in vitro, a recent study has shown a specific NF-κB- and activated protein 1 (AP1)-dependent induction of HBD-2 by probiotic bacteria such as E. coli Nissle 1917 and Lactobacilli [36]. In the same study, normal LPS, probiotic E. coli Nissle LPS, as well as 50 other E. coli strains tested did not induce HBD-2 [36]. The induction of HBD-2 by LPS has been described for the airway epithelia [30] and by indirect IL-1 signaling in skin [37]. The lack of response to LPS in the colon is consistent with the lack of HBD-2 expression in the uninflamed state [38, 39]. Although in vitro studies of HBD-2 suggest an activation by inflammatory agents or inflammation itself [37, 40, 41], the induction of HBD-2 does not necessarily correlate with proinflammatory cytokines such as IL-8 or TNF-α in vivo [38]. Consistent with these findings in the colon, the up-regulation of HBD-2 in gastritis was restricted to that triggered by Helicobacter pylori infection [42]. Future studies will hopefully be able to address these complicated interactions and their regulation in vivo.

DEFENSINS AND LOCALIZATION OF CROHN’S DISEASE: COLONIC EXPRESSION OF DEFENSINS IN CROHN’S COLITIS

Some antibacterial factors such as defensins appear to be induced in Crohn’s disease and ulcerative colitis. Human neutrophil peptides 1–3 as well as lysozyme are expressed in surface enterocytes of mucosa with active IBD but not in

PANETH CELL DEFENSINS

An illustration and hemotoxilin and eosin (HE) staining of Paneth cells in the small intestine are given in Figure 2. HD5 is released as a propeptide from Paneth cells and at least in man, is activated by trypsin in the lumen of the intestinal crypts [44]. The functional significance in bacterial infection has recently been shown in HD5 transgenic mice, which are protected from lethal Salmonella infection [45]. Conversely, matrilysin, which is the relevant protease in mice, may be knocked out genetically, and these deficient mice fail to process defensins efficiently, exhibiting higher bacterial counts and developing intestinal inflammation [46]. Little is known about the regulation of Paneth cell α-defensins. No obvious NF-κB binding sites have been described [26], but because of the lack of an appropriate human cell culture model for Paneth cells, it is hard to study directly the regulation of human Paneth cell defensins. In mice, it has been shown that TLR9 is involved in Paneth cell degranulation [47]. It has been shown in mice that bacteria such as Salmonella are able to downregulate Paneth cell defensin expression [48]. Mice Paneth cell defensins contribute to 70% of total ileal antimicrobial activity, and these ileal peptides are released after stimulation with LPS and other bacterial products [43]. In this context, it might be important to mark the different milieu of colonic and ileal bacterial flora: In the small intestine, where nutrient absorption occurs, the bacterial counts are much lower as compared with the colon, where bacteria are a significant proportion of the luminal content.

Fig. 2. HE staining (A) as well as schematic demonstration (B) of the small intestine. Paneth cells are expressed at the base of the crypts. Paneth cells are the only source of α-defensins HD5 and HD6, which are the major antibacterial factors in the ileum. In mice, Paneth cell defensins contribute to 70% of antibiotic activity [43]. (Figures as published with the permission of Nature Immunology.)
controls [49]. HD5 is stored in a precursor form in normal Paneth cells and is also expressed by metaplastic colonic Paneth cells [50, 51]. The β-defensins show a conspicuous difference between Crohn’s disease and ulcerative colitis in the colon. It has been suggested that HBD-1 is constitutively expressed in the intestinal epithelium [40, 52], and qualitative investigations indeed showed constitutive expression in normal tissue and IBD mucosa [41]. With the quantitative approach, a paradoxical decrease of HBD-1 was found in inflamed mucosa of Crohn’s disease and ulcerative colitis, respectively [38]. Unpublished data from our group also suggest a colonic decrease of HBD-1 in case of patients with a NOD2 mutation, but the mechanism and biological relevance of this observation are still unclear. This decrease of HBD-1, in the case of NOD2 mutation, is comparable with the published decrease in inflamed tissue [38], which could be confirmed in an independent group. However, it remains to be shown that such a decrease in mRNA levels actually translates into the protein level and more importantly, in a diminished mucosal antibacterial activity. The inducible HBD-2, which has been described originally in the skin [23], is also expressed in the colon during inflammation [40], particularly in ulcerative colitis [41]. It has now been shown by various independent groups that HBD-2 is highly induced in inflamed mucosa of ulcerative colitis patients. As compared with ulcerative colitis, this induction is diminished in Crohn’s disease [38, 39, 41]. Most likely, there is a lack of β-defensin induction in Crohn’s disease contributing to a defective antimicrobial barrier, or alternatively, there is an excessive induction in ulcerative colitis. The third defensin studied was HBD-3, which was reported by Harder et al. [24] as a novel, inducible colitis. In Crohn’s ileitis, HBD-3 and -4 could only be found in inflamed ulcerative colitis. In Crohn’s colitis as well as Crohn’s ileitis, the expression of both inducible peptides was diminished or deficient. Furthermore, enterocytes were identified as the source of HBD-3 and -4 expression by in situ hybridization [53]. It is interesting that different inducible β-defensins follow the same pattern, and further studies have to answer the question of the mechanism as well as the functional consequences. A deficiency in the antimicrobial defense systems of defensins may be a reasonable and plausible explanation for the break of the antibacterial barrier function in IBDs.

ILEAL EXPRESSION OF DEFENSINS IN CROHN’S ILEITIS

According to unpublished data, HBD-1 shows a stable ileal expression, which remains unchanged in the case of disease, suggesting that this peptide does not play an important role in case of ileitis. A recent study showed a decreased ileal Paneth cell HD5 and HD6 expression in patients with ileal disease as compared with controls and colonic-nonileal Crohn’s disease. This difference was even more pronounced in patients with a NOD2 mutation, but it is important to point out that the NOD2 wild-type patients already have decreased levels of Paneth cell defensins [54]. The functional significance of HD5 [43] as well as the fact that mouse Paneth cell defensins contribute to ~70% of the antibiotic activity in the ileum [43] illustrate the potential importance of these findings. We could extend these studies recently in surgical specimens from patients from the Cleveland Clinic Foundation (OH). Again, HD5 and HD6 were significantly reduced in Crohn’s ileitis. The Western blot for HD5 showed the same decrease on the protein level (J. Wehkamp et al., manuscript in preparation). In contrast, other Paneth cell products were unaffected in the same patients, suggesting that the number of Paneth cells is unchanged.

CONSEQUENCES FOR Therapy: THE ROLE OF ANTIBIOTICS AND PROBIOTICS

A primary lack in the antimicrobial mucosal barrier in Crohn’s disease would drastically change therapeutic concepts. If a deficiency of these endogenous antibiotics triggered relapse, it would be expected that exogenous antibiotics were an efficacious treatment option. Indeed, antibiotics appear to be effective in Crohn’s disease and similarly, probiotics, such as E. coli Nissle 1917 in ulcerative colitis [1, 55–59]. It is conceivable that the effect of probiotic bacteria is partly a result of the induction of β-defensins, as demonstrated in vitro. In contrast to more than 50 tested E. coli, the probiotic strain E. coli Nissle 1917 and other known probiotic strains such as Lactobacilli and Pedicoccus potently up-regulated HBD-2 expression in intestinal epithelial cells [36]. The induction of HBD-2 is dependent on NF-κB and AP1 activation [36], which is consistent with induction of IL-8 as described recently [60, 61]. This induction of antimicrobial peptides by probiotic bacteria has recently been demonstrated in bees [62], and the same observation in different species suggests a broad and conserved mechanism, which underlines the potential importance of this finding.

CONCLUDING REMARKS

It is remarkable that the main clinical phenotypes, ileal and colonic Crohn’s disease, can be linked to a characteristic lowered defensin profile. Ileal Crohn’s disease is associated with a lack of ileal Paneth cell defensins, which are characteristic of the small intestine. Conversely, colonic Crohn’s disease is associated with a lack of β-defensin induction, which cannot be found in the normal colon. As colon and ileum drastically contrast in the number of Paneth cell defensins, the present hypothesis appears to provide a plausible explanation for disease localization. As compared with other inflammatory cells, which are widely distributed, Paneth cells and their effector molecules (HD5 and HD6) are expressed almost exclusively in the small intestine. So it can be concluded that the small intestine as “the site of crime” with severe inflammation shows an impaired expression of these antibiotic effector molecules. At the same time, it harbors adherent bacteria on the
surface as well as increased bacterial counts in the lumen. The multitude of deficiencies, of which many might still be unknown, other antibiotic peptides, and related transcription factors or transporters leaves enough room for clinical diversity among patients. It should also be determined whether differential expression or mutations in other pattern recognition receptors [63–65] translate into an altered expression of defensins. This understanding of the disease would also drastically change the understanding of therapy. New strategies would try to strengthen the barrier function and protective innate immunity rather than only suppressing the inflammatory secondary response. E. coli Nissle 1917 in addition to Lactobacilli and other known probiotics or even helminths may have the common feature of inducing the antimicrobial peptides, which might be an important feature to help the mucosa to prevent bacterial invasion [36].

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REFERENCES


