

Improvement of a ‘Leaky’ Intestinal Barrier

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Key Words

Intestinal barrier · Mucus · Defensins · Inflammatory bowel disease

Abstract

In Crohn’s disease, the mucus layer appears to be defective in terms of low defensin levels and lack of antibacterial activity. These deficiencies actually explain the Montreal phenotypes and the stable localization of disease in the terminal ileum with low α -defensins from Paneth cells and/or low β -defensins in colonic disease, respectively. Conversely, in ulcerative colitis (UC) the defensin production is normal or even induced, but the mucus layer is thinner and patchy, more in the liquid form and also chemically altered so that antibacterial peptides are not retained and lost into the luminal bacterial bulk. Therefore, both barrier problems allow slow bacterial attachment and invasion, ultimately triggering the massive response of adaptive immunity and tissue destruction. Therefore, leakiness should refer to the antibacterial barrier and not to the general barrier against small molecules, such as mannitol or lactulose, which are not antigenic. The most promising approach in UC seems to be the use of probiotics or the natural compound lecithin as a stabilizer of mucus structure to enhance the barrier. While a phase II study has yielded positive results, the results of the ongoing phase III study are eagerly awaited. It is quite possible that

the protective effect of smoking in UC is related to mucus production in the colon also, but this is not an option. Another alternative would be to shift cell differentiation in the colon towards goblet cell; the relevant differentiation factors are known. In Crohn’s disease, the direct oral application of defensins might be effective if release and binding to the mucus are achieved. In the experimental colitis model, this works quite well. In conclusion, in a situation where enthusiasm about so-called biologics is declining due to loss of response over time, searching for the primary defects in inflammatory bowel disease and treating them may well be worthwhile, although it is unlikely to provide rapid relief.

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Introduction

The intestinal barrier essentially consists of the normally continuous epithelial layer, the cells sticking together by tight junctions and, as a complex secretory product, the mucus layer. The function of this double layer is to remain permeable to allow the passing through of small absorptive molecules like sugars and amino acids while restraining the access of bacteria and possibly bacterial compounds such as LPS. The mucus layer actually consists of 2 strata: a 100 μm layer immediately above and firmly attached to the epithelial cells that is

virtually sterile and on top towards the lumen (and its massive bacterial contamination), which is more liquid in nature and therefore contaminated, another layer of around 700 μm [1]. The minimal bacterial counts directly above the epithelium are not just a consequence of mucus' physical structure but are due to the epithelial secretion of positively charged antibacterial peptides (mostly defensins) binding to various negatively charged mucins (mostly MUC2) [2]. The defensins in the small intestine are mostly produced and secreted by the Paneth cells residing at the bottom of the crypts, in the colon by normal absorptive epithelial and the mucins by goblet cells [3].

Specific Defects in Crohn's Disease

In Crohn's disease, the antibacterial defense appears to be defective with respect to low defensin levels and lack of antibacterial activity. These deficiencies actually explain the Montreal phenotypes of ileal versus colonic disease and the stable localization of disease in the terminal ileum with low α -defensins from Paneth cells and/or low induction of β -defensins in colonic disease, respectively.

In the small intestine, the highest bacterial count is in the terminal ileum due to reflux of colonic contents. In contrast to the colon where Paneth cells appear only as metaplastic cells during inflammation, the small intestine and in particular the lower crypts with their residing stem cells are protected by these antibacterial peptide-producing cells [4]. The major components are human defensin 5 (HD5) and human defensin 6 (HD6), which may either kill bacteria directly or trap them in nets. Inside the Paneth cells, defensin propeptides are stored within granules and released upon bacterial challenge. Most likely in humans, the propeptides are activated by trypsin to the mature defensins. The relevance of these cells is documented by the fact that defective Paneth cells lead to experimental inflammation in the mouse ileum. Genetic variants of human disease synthesize to produce abnormal Paneth cell phenotypes that define subtypes of Crohn's disease [5]. In principle, the expression of both HD5 and HD6 is diminished in ileal Crohn's disease independent of inflammation [6]. Genetic variants in Crohn's disease affecting Paneth cell morphology and function are manifold and include the intracellular receptor for bacterial muramyl dipeptide NOD2, the autophagy gene ATG16L1 and the endosomal stress protein XBP1. Another important mecha-

nism is crinophagy, which leads to the destruction of Paneth cell granules by autophagosomes, as observed in 90% of ileal Crohn's disease patients [7]. In addition, tryptic degradation and protease binding of HD5 has been observed; some patients have mutant HD5 or display Paneth cell necroptosis [8]. Most importantly, new genetic links have been discovered in a dysregulated Wnt-pathway compromising Paneth cell differentiation and thereby function. For this reason, we have suggested the term 'Paneth's disease' for ileal Crohn's disease [9]. Finally, monocytes have recently been demonstrated to enhance the Paneth cell function by Wnt-factors, but this mechanism is defective in monocytes from Crohn's disease patients [10].

In the colon, the regulatory defects in the defensin system are less understood. Here, the constitutive human β -defensin 1 (HBD1) is important besides the inducible partners HBD2 and HBD3 [3]. HBD1 has to be activated by chemical reduction through thioredoxin and is under the regulation of the peroxisome proliferator-activated receptor gamma [11]. In Crohn's disease, HBD1 production is low and the induction of HBD2 and HBD3 is compromised similar to that of another antibacterial peptide, the cathelicidin LL37. Overall, the antibacterial activity of colonic mucosa is lower than normal leading to a barrier defect towards the resident luminal bacterial flora [12].

Specific Defects in Ulcerative Colitis

In ulcerative colitis (UC), the defensin production is normal or even induced [3], but the mucus layer is thinner and patchy, more liquid in nature and also chemically altered so that antibacterial peptides are probably not retained and lost into the luminal bacterial bulk [2]. Therefore, both barrier problems allow bacterial attachment and slow invasion [13], ultimately triggering the massive response of adaptive immunity and tissue destruction. Therefore, the term 'leakiness' should refer to the antibacterial barrier and not the general barrier against small molecules, such as mannitol or lactulose, which are not antigenic.

The mechanisms leading to defective mucus in UC are unclear. We have suggested that the differentiation of goblet cells is compromised in UC. Alternatively, or may be additionally, mucolytic bacteria may actually degrade intact mucins and thus induce a barrier problem. Since mucus is known to retain, store and release defensins, its importance as an antibacterial layer is obvious [2]. As a

consequence, in inflammatory bowel disease (IBD), the mucus is contaminated by bacteria [13]. However, defensin synthesis in UC is even induced as compared to controls. Therefore, these diseases may be interpreted as an adequate inflammatory response to slow bacterial invasion as a consequence of differing barrier defects.

What to Do?

There are several possible and promising approaches to seal the leaky barrier. However, none of these is currently sufficiently effective to reliably induce or maintain remission in most of the patients.

At first sight, the most plausible approach would be the direct attack against resident luminal bacteria by broadband antibiotics. Antibiotics, however, are definitely only known to be effective in special situations like pouchitis or draining fistula. In luminal disease, data are more limited although rifaximine has been shown to be superior to placebo with respect to remission and clinical response in Crohn's disease [14], whereas solid studies on the benefit from antibiotics in ulcerative are not available. In addition, it seems risky to use antibiotics on a permanent basis because of the likely development of bacterial resistance.

Alternatively, certain probiotics known to induce defensin synthesis [15] have been used to maintain remission in UC [16]. For example, the probiotic *Escherichia coli* Nissle has been demonstrated to be equivalent to mesalazine in several studies. Another promising approach in UC seems to employ the natural compound lecithin as a stabilizer of mucus structure to enhance the barrier. A phase II study has been positive [17] and the results of the ongoing phase III study are eagerly awaited. Also, it is quite possible that the known protective effect of smoking

in UC is related to mucus production in the colon, but this is obviously not a reasonable therapeutic option. Another alternative would be to shift cell differentiation in the colon towards goblet cells; here, the relevant differentiation factors are known. Unfortunately, this field has not been investigated thoroughly as a therapeutic tool in experimental models.

In Crohn's disease, direct oral application of natural or modified defensins might be effective if local release and binding to the mucus are achieved. In the experimental colitis model, this works quite well. Since recent data [10] suggest that the genetic defects in the Paneth cell may be bypassed by monocyte-derived Wnt factors, their application may be promising although still very speculative. Moreover, high Wnt activity may unfortunately induce colonic or small intestinal tumors.

Conclusion

In conclusion, in a situation where enthusiasm about so-called biologics is declining due to loss of response over time, searching for the primary defects in IBD and treating them may well be worthwhile. Enhancing the barrier is more likely to help maintain remission than inducing rapid clinical improvement during a severe relapse. Most likely, active disease still will require anti-inflammatory interventions, but for maintenance treatment, a more barrier-directed approach is the most promising way.

Disclosure Statement

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