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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Increased Pancreatic Protease Activity in Response to Antibiotics Impairs Gut Barrier and Triggers Colitis

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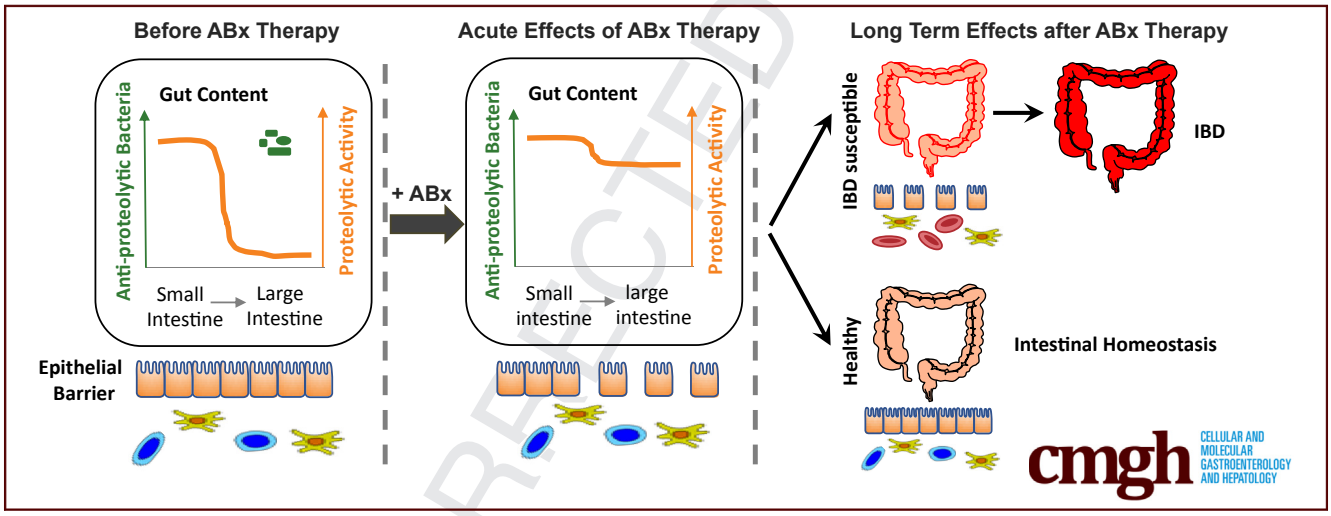

SUMMARY

Antibiotic therapies eliminate antiproteolytic bacteria in the large intestine, resulting in an increase in proteolytic activity, which is detrimental to the epithelial barrier function. In inflammatory bowel disease–susceptible individuals, high proteolytic activity promotes colitis development in the long term.

BACKGROUND & AIMS: Antibiotic (ABx) therapy is associated with increased risk for Crohn's disease but underlying mechanisms are unknown. We observed high fecal serine protease activity (PA) to be a frequent side effect of ABx therapy. The aim of the present study was to unravel whether this rise in large intestinal PA may promote colitis development via detrimental effects on the large intestinal barrier.

METHODS: Transwell experiments were used to assess the impact of high PA in ABx-treated patients or vancomycin/metronidazole-treated mice on the epithelial barrier. Serine protease profiling was performed using liquid chromatography–mass spectrometry/mass spectrometry analysis. The impact of high large intestinal PA on the intestinal barrier in wild-type and interleukin (IL)$10^{-/-}$ mice and on colitis development in IL$10^{-/-}$ mice was investigated using vancomycin/metronidazole with or without oral serine protease inhibitor (AEBSF) treatment.

RESULTS: The ABx-induced, high large intestinal PA was caused by significantly increased levels of pancreatic proteases and impaired epithelial barrier integrity. In wild-type mice, the rise in PA caused a transient increase in intestinal permeability but did not affect susceptibility to chemically induced acute colitis. In IL$10^{-/-}$ mice, increased PA caused a consistent impairment of the intestinal barrier associated with inflammatory activation in the large intestinal tissue. In the long term,

the vancomycin/metronidazole-induced lasting increase in PA aggravated colitis development in IL10$^{-/-}$ mice.

CONCLUSIONS: High large intestinal PA is a frequent adverse effect of ABx therapy, which is detrimental to the large intestinal barrier and may contribute to the development of chronic intestinal inflammation in susceptible individuals. *(Cell Mol Gastroenterol Hepatol 2018;■:■–■; https://doi.org/10.1016/j.jcmgh.2018.05.008)*

Keywords: Epithelial Barrier; Serine Proteases; Gut Microbiota; Inflammatory Bowel Diseases.

Q5 Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, are idiopathic diseases that are thought to be the consequence of a dysregulated intestinal immune response toward the intestinal microbiota.[1-3] IBD are characterized by chronic inflammation, impaired intestinal barrier function, and microbial dysbiosis.[4-7] The highly complex etiology of IBD, comprising approximately 200 susceptibility loci[8] and abundant environmental factors,[9] results in an individual-specific pathogenesis from unknown initial triggers to the manifestation of the chronic inflammation. Interestingly, recent studies suggest a critical role of the microbial ecosystem in the initiation and progression of IBD.[10-13]

The intestinal microbiota of healthy individuals consists of highly diverse communities of microbes performing important functions, such as the production of short-chain fatty acids, metabolism of bile acids,[14] pathogen exclusion,[15] modulation of the intestinal immune system,[16] and degradation of pancreatic proteases in the large intestine.[17] In consequence, antibiotic (Abx)- or infection-induced microbial dysbiosis may exert a significant impact on the onset and progression of chronic metabolic or chronic inflammatory diseases in susceptible organisms.[18-21] Perturbations of the intestinal microbial ecosystem by ABx therapies were found to be associated with functional intestinal disorders, such as irritable bowel syndrome,[22,23] colorectal cancer,[24,25] overweight, and asthma.[26] In the context of IBD, several clinical studies have already revealed that early and frequent ABx therapies, especially metronidazole or fluoroquinolone treatments, are associated with increased risk for Crohn's disease.[27,28] However, the causal role of ABx therapies in the disease development and the mechanisms underlying this potential serious long-term adverse effect of ABx on the intestinal immune homeostasis remain unknown.

Interestingly, it has long been known that ABx treatments can result in increased luminal serine protease activity (PA) in the large intestine of rodents[29,30] or patients.[31,32] This increase in PA is assumed to be caused by the ABx-mediated eradication of yet unknown intestinal bacteria that normally inactivate the high load of pancreatic proteases on entry of the chyme into the large intestine.[33,34] The finding that *ex vivo* exposure of murine colonic mucosa to high PA fecal supernatants from patients with diarrhea-predominant irritable bowel syndrome can induce a serine protease-dependent increase of the mucosal permeability[35] indicates that the ABx-mediated rise in PA may be rather detrimental to the large intestinal barrier. In the context of IBD, reduced intestinal barrier functions and increased translocation of luminal antigens into the mucosal tissue are known to promote chronic inflammation.[36-39] In view of these data, we hypothesized that the ABx-increased PA in the large intestine is a relevant risk factor for the development of colitis in susceptible organisms.

Methods

Ethics Statement

The breeding and experimental use of mice in the animal facilities of the Technische Universität München (School of Life Sciences Weihenstephan) was approved by the local institution in charge (Regierung von Oberbayern; approval number 55.2-1-54-2531-99-13 and 55.2-1-54-2532-17-2015).

Stool Sample Collection From Patients

Samples were collected before and after ABx therapy at the Capio Hospital General de Catalunya in Barcelona[40] and the Medical University of Graz (ethical approval number 17-199 ex 05/06) and stored at -20°C until analysis. Patient characteristics are summarized in Table 1.

Experimental Design (Wild-Type and Interleukin 10$^{-/-}$ Mice)

In all experiments, mice were fed with vancomycin/metronidazole (V/M)-containing chow mash or control (ctr) mash *ad libitum* for the indicated period of time. V/M mash was prepared by mixing a vancomycin (0.25 g/L; Fluka)/metronidazole (1.0 g/L; Sigma) solution with chow powder (ssniff-Spezialdiäten GmbH) in a 1:1 volume (mL)/weight (g) ratio. Q6

In the short-term experiments, 8-week-old wild-type (WT) (C57BL/6, male and female) or interleukin (IL) 10$^{-/-}$ mice (129/SvEv, male and female) (n ≥5/group) were treated with V/M for either 2 days, 7 days, or left untreated. The protease inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (300 mg/kg body weight) (AEBSF; Sigma) or water was applied by oral gavage starting from 1 day before V/M treatment where indicated. Fresh fecal pellets were sampled daily from all mice. Fluorescein isothiocyanate dextran (4 kDa; Sigma) was applied by oral gavage 4 hours before mice were sacrificed by CO_2.

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Abbreviations used in this paper: ABx, antibiotic; AEBSF, 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride; cecal-sup, cecal-supernatants; ctr, control; DSS, dextran sulfate sodium; GF, germ-free; IBD, inflammatory bowel diseases; IL, interleukin; LC-MS/MS, liquid chromatography–mass spectrometry/mass spectrometry; PA, protease activity; PBS, phosphate-buffered saline; PMSF, phenylmethane-sulfonylfluoride; stool-sup, stool-supernatants; SPF, specific pathogen-free; TEER, transepithelial electrical resistance; V/M, vancomycin/metronidazole; WT, wild-type.

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