Bacterial proteases in IBD and IBS
Natalie Steck, Kerstin Mueller, Michael Schemann, Dirk Haller

ABSTRACT
Proteases play a decisive role in health and disease. They fulfill diverse functions and have been associated with the pathology of gastrointestinal disorders such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). The current knowledge focuses on host-derived proteases including matrix metalloproteinases, various serine proteases and cathepsins. The possible contribution of bacterial proteases has been largely ignored in the pathogenesis of IBD and IBS, although there is increasing evidence, especially demonstrated for proteases from pathogenic bacteria. The underlying mechanisms extend to proteases from commensal bacteria which may be relevant for disease susceptibility. The intestinal microbiota and its proteolytic capacity exhibit the potential to contribute to the pathogenesis of IBD and IBS. This review highlights the relevance of host- and bacteria-derived proteases and their signalling mechanisms.

INTRODUCTION
Inflammatory bowel disease (IBD)—including the two main distinct pathologies ulcerative colitis (UC) and Crohn’s disease (CD)—and irritable bowel syndrome (IBS) are chronically relapsing diseases with an accelerating incidence, especially in developed countries. It is noteworthy that IBS-like symptoms such as diarrhoea or visceral pain are frequently observed in some patients with IBD in remission. Although IBD and IBS are two distinct entities, their complex pathogenesis involves common features including alterations in immune responses, altered microbiota composition with an impact on microbiota-host interactions, impaired intestinal barrier functions, altered bowel habits and changes in visceral sensitivity. There is increasing evidence that all of the above factors are influenced by proteases, although the particular protease involved and the pathways influenced by proteases may be different in IBD and IBS. This review focuses on the involvement of host- or bacterial-derived proteases in IBD or IBS and highlights those protease-activated pathways that may be relevant in the pathogenesis of IBD and IBS.

Host-derived proteases in IBD and IBS
The large group of zinc-dependent matrix metalloproteinases (MMPs) plays a central role in extraacellular matrix turnover. Furthermore, MMPs proteolytically activate a variety of non-matrix substrates such as cytokines, chemokines, growth factors and junction proteins. The deregulated expression or activity of host-derived MMPs has been implicated in several diseases including arthritis, atherosclerosis and colon cancer.

Increasing evidence suggests that MMPs are the predominant proteases involved in the pathogenesis of IBD. MMPs influence the disease progression in multiple ways involving the function and migration of inflammatory cells as well as matrix deposition and degradation. The expression and activity of certain MMPs is increased during acute inflammation, but also an imbalance between MMPs and their natural tissue inhibitors (TIMPs) has been reported for IBD. Besides MMPs, other proteases including trypsin, neutrophil elastase, mast cell tryptase, cathepsins and thrombin have been implicated in IBD. Supernatants of mucosal biopsy specimens from patients with IBS evoked activation of visceral nociceptive neurons as well as enteric neurons and induced hyperalgesia, a key feature of IBS. The responses could be attributed to serine proteases.

Intestinal microbiota in IBD and IBS
Changes in diversity and composition as well as functionality of the intestinal microbiota were associated with IBD and IBS. Pathogenic infections are suggested as the starting point for IBD and IBS, but further work is needed to understand the role of commensal bacteria and their possible contribution to the pathogenesis of intestinal disorders. In the context of intestinal inflammatory disease, it has to be considered that commensalism changes into a harmful situation for the host which then becomes a continuous inflammatory trigger.

Protease activity in the gut lumen
Excessive concentrations of proteases have been found in the faeces of patients with UC or IBS. Consistent with these findings, secreted factors of colonic biopsy samples from patients with IBD and IBS showed increased proteolytic activity. Although the origin of the proteolytic activity was not elucidated, it is conceivable that proteases released from biopsy specimens are derived from the host. However, increased faecal proteolytic activity might result from colonic luminal bacteria which release serine, cysteine and metalloproteases. Oral antibiotic treatment of mice resulted in reduced numbers of colonic microbiota and reduced colonic luminal serine protease activity, which provides further evidence for the bacterial origin of the proteases. Bacterial proteolytic activity could be demonstrated even in the absence of inflammation, supporting the hypothesis that bacterial proteases are ubiquitously present in the gut lumen but only have an impact in a susceptible situation.
IMPLICATIONS FOR BACTERIAL PROTEASES IN THE PATHOLOGY OF IBD
Role of pathogen-derived proteases
It has been suggested that alterations in microbial diversity and composition in disease-susceptible populations may alter innate defence mechanisms leading to chronic immune-mediated activation in active IBD.29–32 Pathogens can be considered as the initiation step at the beginning of the disease development or during disease progression.18 20 31 Many attempts have been made to identify the bacterial structures or molecules responsible for the pathogenicity of microbes. Besides the mechanism of type-specific secretion systems which allow the infiltration of bacterial material or whole bacteria into host cells, proteases have been shown to be involved in the infectious process of pathogens. However, the pattern of different types of proteases and their expression, regulation, activation and substrate specificity is very diverse. Host tissue provides different target points for bacterial proteases. In addition to the activation of specific types of host receptors, which will be discussed later, the degradation of extracellular matrix and the disruption of epithelial barrier function exhibits the most frequently described consequence of bacterial proteases. Targeting host epithelial barrier function is a central mechanism to be considered in the complex pathogenesis of IBD. Impaired intestinal barrier function has been associated with IBD,32 although it is still uncertain whether the loss of barrier integrity is the cause or a consequence of chronic inflammation. Table 1 the proteases from pathogens targeting epithelial or endothelial barrier function in different organs and summarises the general mechanisms attributed to bacterial proteases which might be relevant in IBD.

Role of commensal-derived proteases
The adherence junction protein E-cadherin is the best described target for bacterial proteases derived from pathogens. This might be surprising as E-cadherin provides cell–cell contact on the lateral site of epithelial cells and it remains unclear how bacterial proteases in the gut lumen get access to their cleavage sites. We have demonstrated for the first time that a commensal-derived protease plays a role in intestinal inflammation. Gelatinase (GelE), a zinc-dependent metalloproteinase from Enterococcus faecalis, disrupted the integrity of the intestinal epithelial barrier by targeting the junction proteins occludin and E-cadherin.44 The presence of disease susceptibility, but not tissue inflammation, is required for E faecalis GelE-mediated disruption of colonic barrier function (figure 1), supporting the hypothesis that commensal-derived proteases remain harmless in the healthy host. Similar to pathogens, commensal bacteria also possess a variety of virulence genes which are expressed under certain environmental conditions. E faecalis GelE can be regarded as an example in which a commensal-derived protease that has been associated with bacterial virulence plays a role in the development of intestinal inflammation. Further analysis of the structure/function interplay between bacteria and the host, including the

Table 1  Bacterial proteases target epithelial cell barrier function

<table>
<thead>
<tr>
<th>Species</th>
<th>Classification</th>
<th>Protease</th>
<th>Host target structure/proposed mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus anthracis</td>
<td>Pathogen</td>
<td>Metalloproteinase lethal toxin (LT)</td>
<td>LT impairs barrier function in primary human endothelial cells (altered VE-cadherin distribution)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M4 metalloproteinase neutral protease (Npr599)</td>
<td>Npr599 and InhA reduce endothelial barrier function through increased syndecan-1 ectodomain shedding in cultivated murine mammary gland cells</td>
<td>36</td>
</tr>
<tr>
<td>Citrobacter rodentum</td>
<td>Pathogen</td>
<td>Lyphostatin: virulence factor consisting of a glycosyltransferase, a protease and an aminotransferase</td>
<td>Disruption of epithelial barrier function via modulation of the small GTPase Rho and Cdc42</td>
<td>37</td>
</tr>
<tr>
<td>Clostridium difficile, C. sordelli, C. novyi</td>
<td>Pathogen</td>
<td>Large clostridial toxins (glycosyltransferases)</td>
<td>Inactivation of GTases Rh, Rac and Cdc42 in intestinal epithelial cells</td>
<td>28</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>Opportunistic pathogen</td>
<td>Collagenase A</td>
<td>Intestinal barrier function, basal type-IV-collagen, mucus</td>
<td>38</td>
</tr>
<tr>
<td>Enterohaemorrhagic Escherichia coli</td>
<td>Pathogen</td>
<td>Metalloproteinase StcE</td>
<td>Cleavage of mucin 7 and glycoprotein 340, facilitation of adherence to epithelial-like HEP-2 cells</td>
<td>39</td>
</tr>
<tr>
<td>Enterotoxigenic Bacteroides fragilis</td>
<td>Opportunistic pathogen</td>
<td>Metalloproteinase fragilisin or B fragilis toxin (BFT)</td>
<td>Induction of γ-secretase dependent shedding of E-cadherin ectodomain in HT29 cells</td>
<td>40</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>Pathogen</td>
<td>Serine protease Helicobacter pylori high temperature requirement A (HptHtrA)</td>
<td>Reduction of epithelial barrier integrity through targeting E-cadherin</td>
<td>41</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Pathogen</td>
<td>Pseudomonas elastase</td>
<td>Reduction of barrier function in MDCK cells, altered ZO-1 expression and disturbed microfilaments</td>
<td>42</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Opportunistic pathogen</td>
<td>Serine proteases</td>
<td>Modulation of chemokine expression through NF-xB activation</td>
<td>43</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>Pathogen</td>
<td>Metalloproteinase haemagglutinin/</td>
<td>Reduction of barrier integrity through actin and tight junction rearrangement</td>
<td>44</td>
</tr>
</tbody>
</table>
were used for intestinal inflammation. Interleukin-10-deficient (IL-10–/–) mice are genetically-driven spontaneous mouse models for the development of chronic inflammation in the colon and ileum, respectively. Rag2-deficient (Rag2–/–) mice lack B and T cells and develop colitis after transfer of CD4+ T cells. NOD2-deficient (NOD2 Δ/Δ) mice lack the intracellular pattern recognition receptor NOD2 (nucleotide-binding oligomerisation domain 2) and are susceptible to certain bacterial infections. Appropriate wild type (Wt) counterparts 129SvEv and C57B6 were used as controls. Except for T cell mice lack the intracellular pattern recognition receptor NOD2 (nucleotide-binding oligomerisation domain 2) and are susceptible to certain bacterial infections. Appropriate wild type (Wt) counterparts 129SvEv and C57B6 were used as controls. Except for T cell mice, all other models did not develop histological changes with respect to inflammation in the colon after 8 weeks. Distal colon segments of the mice were apically stimulated with purified GelE (10 μg/ml) for 5 h in Ussing chamber systems. The results are expressed as the percentage change in transepithelial electrical resistance (TER) compared with the initial value of the tissue. Data are partially published44 and represent median with 25th and 75th percentiles from five animals per group; *p≤0.05.

**PROTEOLYTIC ACTIVATION OF NEURONS**

The functional relevance of the elevated protease levels in patients with IBD and IBS has been demonstrated. In a rodent model, faecal supernatants of diarrhoea-predominant IBS patients with elevated serine protease contents induced visceral hypersensitivity and increased paracellular permeability via the protease-activated receptor (PAR) 2.26 However, faecal supernatants of patients with UC generated hyposensitivity to rectal distension, which was a result of PAR4 activation by cathepsin G. This anti-nociceptive action overpowered the pro-nociceptive effects of PAR2 activating proteases in the faecal supernatants.54 This study suggested that even though patients with IBD and IBS have increased faecal protease levels, the functionally active proteases must be different and, in addition, signal through distinct mechanisms. Supernatants of colonic biopsy samples of patients with IBS released proteolytic mediators that directly sensitised murine sensory neurons and generated visceral and somatic hypersensitivity through the activation of PAR2.16 Further studies showed that mucosal biopsy supernatants from patients with IBS also activated human enteric neurons52 as well as rat visceral nociceptive neurons.54 In both studies proteases have been identified as the main mediator responsible for neural excitation. Serine proteases such as thrombin, trypsin or mast cell tryptase, which are released by epithelial cells, immune cells, blood cells or even neurons, activate enteric and visceral sensory neurons as well as enteric glia by targeting PARs.50 51 There are four PARs (PAR1, PAR2, PAR3, PAR4), all of which are G-protein coupled tethered ligand receptors. They are activated by the proteolytic cleavage of the N-terminus which allows the tethered ligand to bind to the receptor. PAR1, PAR2 and PAR4 activate enteric neurons (see below for further details). However, PAR4 inhibits excitability of dorsal root ganglion neurons which supply the sensory innervation of the gut.52 This effect may explain the finding that PAR4 exerts analgesic effects by suppressing somatic and visceral hyperalgesia and pain.53 54 The functional expression of PARs in different cells may have a clinical impact in that it may open new ways to specifically target protease actions. In this context, it is important to realise
that the contribution of PARs is different between rodent and human enteric neurons. While submucous neurons in the guinea pig have strong responses to PAR2 activation, PAR1 seems to be the dominant receptor in human submucous neurons. It needs to be considered that PARs are present on muscle cells, epithelial cells and immune cells as well as on neurons. For example, human tissue resident macrophages are activated by PAR2 ligands. Therefore, the disease relevant PAR signalling is a combination of several neuronal and non-neuronal pathways. It is not known whether the neural- and glia-mediated actions of proteases are part of the cascades initiating or maintaining inflammatory processes or whether they serve protective functions. In principle, bacterial proteases may also be able to activate nerves in the gastrointestinal tract but this has not yet been demonstrated. For the purpose of this review, we therefore addressed this open issue and found that the metalloproteinase GelE from commensal Enterococcus faecalis activated about 20% of guinea-pig enteric neurons (figure 2). Interestingly, pretreatment of the tissue with GelE negatively influenced the ability of a selective PAR2-activating peptide to excite neurons. This suggests that GelE may signal through PAR2-expressing or dependent pathways, but further evidence for such a route is needed.

HOST RECEPTORS FOR BACTERIAL PROTEASES

Protease-activated receptors

The potential of different bacterial proteases to target PAR as well as other host receptors has been shown for respiratory and oral epithelial cells as well as for platelets and neutrophils (table 2). However, the mode of action of bacterial proteases in the gastrointestinal tract, except for E faecalis GelE, is still largely unknown. In general, proteases target the G-protein coupled PAR receptors and induce activation by proteolytic cleavage of the N-terminal ligand or inactivation by disarming the receptor due to alternative cleavage of the N-terminus. The bacterial arginine-specific cysteine protease gingipain-R of the periodontitis pathogen Porphyromonas gingivalis has been shown to cleave and activate PAR2 on human neutrophils, PAR1 and PAR4 on platelets to induce platelet aggregation and PAR1 and PAR2 on an oral epithelial cell line to induce secretion of the pro-inflammatory cytokine interleukin 6 (IL-6). In addition to PARs, several other cell surface proteins such as occludin and E-cadherin were targets of gingipain in Madin-Darby canine kidney cells and CD14 human monocytes. The lung opportunistic pathogen Pseudomonas aeruginosa secreted the elastolytic metalloproteinase P aeruginosa elastase/LasB which disarmed PAR2 by proteolysis, made the receptor unresponsive to activating proteases and avoided receptor internalisation and mobilisation of intracellular pools. However, P aeruginosa elastase has also been shown to increase respiratory epithelial permeability by cleavage of tight junction proteins zonula occludens 1 and 2. Furthermore, P aeruginosa released the exoprotease LepA which induced the activation of the transcription factor NF-kB via PAR1, PAR2 and PAR4 activation. Serratia marcescens, which is an opportunistic pathogen of the respiratory and urinary tract,
produced the zinc metalloproteinase, serralysin, which has been shown to induce IL-6 and IL-8 mRNA expression in a respiratory cell line and transactivation of AP-1, C/EBP- and NF-kB-driven promotors via PAR2. A house dust mite cysteine protease allergen (Der p1) has been shown to target PAR2 but to inactivate PAR1 in respiratory epithelial cells and to induce release of pro-inflammatory cytokines. The mite allergens Der p3 and Der p9, which are serine proteases, activated lung epithelial cells by interaction with PAR2 and induced the release of pro-inflammatory cytokines.

**Pattern recognition receptors**

Toll-like receptors (TLR) are part of the innate immune system and belong to the pattern recognition receptor family that identifies microbial pathogens through the recognition of pathogen- or microbial-associated molecular patterns (carbohydrates, nucleic acids, peptidoglycans, lipoteichoic acids, lipoproteins). Direct proteolytic activation of a full-length TLR has recently been described for the avian TLR15. Virulence-associated microbial-derived proteases from the fungi *Candida guilliermondii*, *Trichosporon* spp., *Penicillium* spp. and *Mucor* spp. and the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* have been shown to activate TLR15, which is a unique type of receptor that combines TLR characteristics with an activation mechanism typical of the evolutionary distinct PAR. Although it is still unknown which proteases activate TLR15, one could speculate that these are serine proteases as the receptor activation could be inhibited by the serine protease inhibitor phenylmethanesulfonylfluoride (PMSF). Mammalian seem to lack a TLR that is able to sense proteolytic activity, although mammalian TLR4 can be activated by elastase-activated compounds from the extracellular matrix. Furthermore, lipopolysaccharide (LPS) recognition by TLR4 was indirectly influenced by trypsin which is augmented in ileal inflammation and cleaved MD-2, an accessory glycoprotein essential for TLR4 signalling. The proteolysis of MD-2 provided a mechanism for intestinal epithelial LPS tolerance that helped to regulate immune responses to commensal bacteria-derived ligands. Recent work connected TLR and PAR signalling and described distinct features of the TLR–PAR interaction which contribute to signal diversity in inflammation and host antimicrobial responses. The cross-talk between different receptor signalling cascades and the recognition of diverse microbial signals provide homeostasis, but further investigations are needed to understand this complex network more fully.

**E-cadherin**

The adherence junction protein E-cadherin is a calcium-dependent single-pass transmembrane protein generally expressed in the lateral plasma membrane of epithelial cells. E-cadherin provides contact to adjacent cells and plays a major role in epithelial cell differentiation. The intracellular domain is highly conserved and signals through cytoplasmic proteins from the catenin family. The extracellular domain could act as a receptor for bacterial or fungal entry into epithelial cells, which so far has only been demonstrated for surface proteins. *Intimin A* from *Listeria monocytogenes* and invasion Als3 from *Candida albicans* interact with E-cadherin and mediate the internalisation of the respective microorganisms. E-cadherin is also targeted by bacterial proteases; the metalloproteinase toxin BFT from *Bacteroides fragilis* was shown to induce the shedding of the E-cadherin ectodomain through an unknown intestinal epithelial cell receptor-mediated induction of γ-secretase. A direct E-cadherin cleavage could be demonstrated for the trypsin-like serine protease HprH of *Helicobacter pylori*. We demonstrated that a commensal-derived metalloproteinase, GeLP from *E. faecalis*, was able to degrade the extracellular domain of E-cadherin in the susceptible host, suggesting that proteases from the commensal gut microbiota might play a role in the disease pathogenesis of chronic intestinal inflammation, but only if they get access to the target tissue under predisposed conditions. The proteolytic cleavage of E-cadherin, either direct or
indirect, reflects an important mechanism for bacteria—especially pathogens—to reach the intercellular space and to translocate across the epithelium. Furthermore, E-cadherin is targeted by a number of endogenous metalloproteinases including stromelysin-1, matrilysin, ADAM-10 and meprin-β.

Protease-dependent receptor activation

The release of tumour necrosis factor α (TNFα) is associated with an increased permeability of the gut epithelial barrier. The blockade of TNF with anti-TNF antibodies is an established strategy in the treatment of IBD. The increase of soluble biologically active TNF arises from the conversion of membrane-bound TNF by TNF-converting enzyme (TACE). TACE or ADAM17 belong to the ADAM (a disintegrin and a metalloprotease) family of metal-dependent proteases and has been additionally implicated in the shedding of other membrane-bound precursors of cytokines and growth factors.

One of these factors is transforming growth factor-β (TGF-β) which in turn activates the epidermal growth factor receptor (EGFR). Phosphorylated EGFR induces the activation of mitogen activated protein kinases and mediates changes in intestinal permeability. Many of these processes and receptor activation mechanisms are described in the context of carcinogenesis, a frequent IBD-associated complication. In particular, the fact that numerous cytokines and growth factors are membrane-associated as precursors and require proteolytic conversion may represent a novel mechanism for bacterial-derived proteases.

CLINICAL IMPLICATIONS

The inhibition of host-derived proteases has been discussed as a therapeutic option in IBD but there are only limited reports with inconsistent results. Studies in animal models showed that inhibition of cathepsins and tryptase ameliorates chemical-induced colitis. Furthermore, the serine protease inhibitor camostat has been reported to induce and maintain remission in patients with UC. Various MMP inhibitors have been shown to improve inflammation, particularly in rat models of IBD but also in dextran sodium sulfate (DSS)-induced colitis in mice. Despite the fact that MMP inhibitors showed beneficial therapeutic effects in...
certain susceptibility is necessary for the involvement of commensal-derived proteases in IBD. Adverse side effects, presumably resulting from non-specific actions and/or broad spectrum inhibition of host proteases also affecting functions of other organs, need to be addressed before protease inhibitors become a therapeutic option. In addition, the use of endogenous protease inhibitors could be considered as a treatment option in IBD to avoid adverse side effects and to reconstitute the proteolytic balance in the gut. One example is elafin, a serine protease inhibitor, the expression of which is reduced in the gut mucosa of IBD patients. Furthermore, it has been demonstrated that elafin protects against DSS-induced colitis through the inhibition of pro-inflammatory mediators and the strengthening of epithelial barrier function.

The inhibition of PARs by PAR antagonists could be an effective strategy to suppress protease-mediated action, particularly if they specifically target a particular PAR. The use of PAR antagonists as a treatment option for IBD and IBS is supported by their involvement in the generation of pain, inflammation, increased permeability, as well as motor and secretory disorders in the intestine. In this respect it may even be beneficial to develop PAR agonists which would provide the PAR4-mediated anti-nociceptive action in humans.

A few clinical trials have shown that the administration of probiotics such as the probiotic mixture VSL#3 improved IBS symptoms including pain/discomfort, distension/bloating and defecation and induced and maintained remission in patients with UC. Furthermore, the feeding study conducted by Kunze et al provided evidence for the interaction between the probiotic Lactobacillus reuteri and colonic enteric neurons. Although probiotic efficacy could be demonstrated in IBD and IBS, the active functional components/structures, the molecular mechanisms or the primary probiotic target still remain to be clarified. It may be speculated that the protective role of probiotics may be related to the release of proteases and/or protease inhibitors. Such a mechanism has recently been reported for the serine protease lactocepin from Lactobacillus paracasei which inhibited the recruitment of pro-inflammatory cells to the site of inflammation through degradation of chemokines. The anti-inflammatory effect of lactocepin suggested that bacterial proteases play an important role in the regulation of intestinal homeostasis.

**Key messages**

- Host-derived proteases are involved in the pathogenesis of IBD and IBS.
- Protease-activated receptors are specific targets for host-derived and bacteria-derived proteases.
- Proteases belong to virulence factors of intestinal pathogens and commensals.
- Mechanistically, bacterial proteases target and impair barrier function of epithelial cells and activate enteric neurons.
- Certain susceptibility is necessary for the involvement of commensal-derived proteases in IBD.

**REFERENCES**


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