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Mechanisms of Disease: protease functions in intestinal mucosal pathobiology

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SUMMARY

Of all our organ systems, the gastrointestinal tract contains the highest levels of endogenous and exogenous proteases (also known as proteinases and peptidases); however, our understanding of their functions and interactions within the gastrointestinal tract is restricted largely to nutrient digestion. The gut epithelium is a sensor of the luminal environment, not only controlling digestive, absorptive and secretory functions, but also relaying information to the mucosal immune, vascular and nervous systems. These functions involve a complex array of cell types that elaborate growth factors, cytokines and extracellular matrix (ECM) proteins, the activity and availability of which are regulated by proteases. Proteolytic activity must be tightly regulated in the face of diverse environmental challenges, because unrestrained or excessive proteolysis leads to pathological gastrointestinal conditions. Moreover, enteric microbes and parasites can hijack proteolytic pathways through 'pathogen host mimicry'. Understanding how the protease balance is maintained and regulated in the intestinal epithelial cell microenvironment and how proteases contribute to physiological and pathological outcomes will undoubtedly contribute to the identification of new potential therapeutic targets for gastrointestinal diseases.

Keywords

enteric pathogen; inflammation; intestinal permeability protease; protease activated receptor

INTRODUCTION

Protease genes constitute about 2% of the mammalian genome and their gene products are recognized as enzymes vital for processes as diverse as development, coagulation, cell death, inflammation and immunity.¹ Proteases, which are also known as proteinases and

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peptidases, catalyze the cleavage of proteins through the hydrolysis of peptide bonds. The mechanism of hydrolytic cleavage used allows the proteases to be divided into several different families: threonine, aspartate, serine, and cysteine proteases, and metalloproteinases.²

Historically, proteases were considered to be largely degradative enzymes that were associated primarily with the breakdown of proteins and protein turnover. The broader scope of protease actions emerged with the concept of 'limited proteolysis', a term coined by Linderstrom-Lang, which distinguished the precise specificity of peptide bond cleavage from the nonspecific proteolysis associated with protein degradation.³ Limited proteolysis is increasingly recognized to have a vital role in the regulation of signaling processes important for intestinal epithelial cell (IEC) function.

The proteases that affect the gastrointestinal tract can be luminal, circulating, secreted, intracellular, intramembrane or pericellular enzymes. Gastrointestinal function is dependent on pericellular proteolysis, as both the apical and basolateral membranes of IECs are continually exposed to high levels of proteases from many cell sources (Figure 1). Conversely, proteases elaborated by IECs regulate their environment, including remodeling of the extracellular matrix (ECM).⁴ There is growing evidence that intestinal proteases have a role in the pathogenesis of gastrointestinal inflammatory, autoimmune and neoplastic diseases; however, our current understanding of the impact of proteases on gastro intestinal pathophysiology is far from complete.

REGULATING THE PROTEASE BALANCE

Such is the enormity of proteolytic potential that proteolytic activity, by necessity, must be tightly regulated to prevent inappropriate and frequently destructive proteolysis. There are numerous mechanisms in the gastrointestinal tract that are needed to achieve a protease balance.

Synthesis as inactive zymogens

Most proteases are synthesized as inactive, latent protease precursors (zymogens) that have to undergo proteolytic cleavage or some other event to be activated. Serine proteases are well known for being activated via zymogen cascades, the simplest of which involves at least two consecutive proteolytic reactions, with one protease zymogen being the substrate for an active protease.⁵

The hallmark of a protease cascade is that a signal can be specifically and irreversibly amplified each time a downstream zymogen is activated, unleashing a burst of proteolytic potential. This process is illustrated by nutrient digestion, in which pancreatic trypsinogen, a serine protease zymogen, is released into the lumen of the small intestine and activated to become trypsin by enteropeptidase (originally known as enterokinase), a membrane-spanning serine protease that is located in the IEC brush border.⁶ In turn, trypsin activates pancreatic chymotrypsinogen, procarboxypeptidases, proelastases, and prolipases in a classic two-stage cascade.⁵ Significant tissue destruction can occur when protease cascades are prematurely activated. Premature activation of trypsin in the pancreas or pancreatic ducts promotes uncontrolled proteolysis and autodigestion, which are features of pancreatitis and cystic fibrosis.⁷

Protease cascades do not necessarily act in isolation. Sometimes, multiple protease families combine to form protease networks, such as occurs during cell migration and ECM degradation.¹ For example, conversion of some ECM-degrading matrix metalloproteinase (MMP) zymogens to active enzyme forms is accomplished by serine proteases of the

plasminogen activator system,⁸ and others by furin-like prohormone convertases.⁹ The complexity of protease systems and the magnitude of proteolytic activity that can be generated by protease networks are largely underestimated.

Spatial and temporal compartmentalization

The access of enterocyte proteases to their appropriate substrates is further regulated by spatial and temporal compartmentalization. For example, the mast-cell proteases (tryptase, chymase and granzyme B) are compartmentalized within intracellular granules and released in response to inflammation associated with stress-induced enteropathies, allergy, nematode infection and reperfusion injuries.¹⁰

Localization of proteases in the pericellular environment can occur through both direct and indirect mechanisms. MMP-14 (also known as membrane-type-1 matrix metalloprotease),⁴ matriptase (an epithelial type II transmembrane serine protease; also known as MTSP-1)^{11,12} and dipeptidyl peptidase 4 (DPP IV; also known as CD26)¹³ are all tethered directly to the plasma membrane by a membrane-spanning domain, which anchors proteolysis to the cell surface. Other proteases, such as the secreted plasminogen activators, are sequestered within the pericellular environment by being bound to specific cell-surface receptors, thereby increasing the efficiency of plasminogen activation and subsequent plasmin-dependent proteolysis.¹⁴ The concentration of protease activity within pericellular microenvironments is crucial for angiogenesis, inflammation, and cell migration in the gastrointestinal tract.

Termination of activity

The termination of IEC proteolytic activities is also vital and is accomplished by specific endogenous inhibitors and clearance receptors. MMP activity is regulated by a group of endogenous inhibitory proteins known as tissue inhibitors of metalloproteinases (TIMPs).⁴ Serpins, a large family of serine protease inhibitors, target multiple steps within serine protease cascades by acting as irreversible 'suicide substrates'.¹⁵ Inhibition of fibrinolysis, for example, can occur at the level of plasminogen activators (through the actions of plasminogen activator inhibitors PAI-1 and PAI-2) or at the level of plasmin (by α_2 -antiplasmin). Adding to this complexity, serpins can be cleaved by other proteases, such as MMPs, which neutralizes their protease inhibitory activity. Protease-inhibitor complexes are recognized and rapidly cleared by specific scavenger receptors. The family of low density lipoprotein (LDL) receptors have a prominent role in the clearance of these complexes and also participate in modulating signaling responses that inform the cell of changes in the pericellular environment.¹⁶

PROTEASES AS SIGNALING MOLECULES

Proteases released into the extracellular space modulate IEC behavior through autocrine, paracrine, and endocrine modes of action. Proteolytic cleavage governs many multicomponent signaling events. These events include the regulation of growth-factor activation and availability, proprotein maturation, enzyme activation, shedding of cell surface receptors, and ECM degradation and turnover. In contrast to most other types of signaling event, protease-mediated signaling is usually irreversible. Numerous regulatory factors important to gastrointestinal physiology are proteolytic substrates, including epidermal growth factor (EGF), transforming growth factor β (TGF- β), insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), trefoil factors (TFF), colony-stimulating factors (CSFs), hepatocyte growth factor (HGF; also known as scatter factor), platelet-derived growth factor (PDGF), tumor necrosis factor (TNF) family members, interleukins (ILs) and interferons.

Proteases can participate in inter-receptor signal transmission through transactivation of growth factor receptors. For example, members of the ADAM (a disintegrin and metalloproteinase domain) family of proteases, which are activated by G-protein-coupled receptors, liberate membrane-tethered growth factors, which then activate the epidermal growth factor receptor.^{17,18} Proteases can generate molecular fragments from endogenous proteins that can stimulate autoimmune responses; however, this function has not yet been demonstrated in the gastrointestinal tract. Both the immediate effects of substrate cleavage and the downstream consequences of secondarily activated signaling pathways must, therefore, be considered when analyzing the consequences of a specific protease activity (Figure 2).

Proteolytic activation of G-protein-coupled cell-surface receptors, known as proteinase-activated receptors (PARs), is directly implicated in the control of many gastrointestinal physiological activities.^{19–22} PARs are activated by proteolytic cleavage of their amino-terminal domains, which exposes a new amino terminus that acts as a tethered ligand that binds to and activates the receptor.²³ Serine proteases present in the intestinal lumen and elaborated by several cell types of the intestinal mucosa (e.g. neurons, fibroblasts and immune cells) can activate PARs²⁰ and, therefore, are potential endogenous agonists for PAR activation. PAR1, PAR3, and PAR4 are activated by thrombin. Mouse PAR3 is a cofactor required for the activation of PAR4 by thrombin.²⁴ PAR2 is activated by several trypsin-like serine proteases, including trypsin and mastcell tryptase.^{25,26} Other proteases, for example, elastase and chymase,^{27–29} cleave PARs downstream of the amino-terminal tethered ligand sequence, thereby preventing further proteolytic activation and abolishing PAR signaling.

Activation of PAR1 or PAR2 alters epithelial and smooth-muscle functions in both the small intestine and colon. Although PAR3 and PAR4 are present in the gastrointestinal system,³⁰ few effects of these PARs on gut functions have been reported. PARs are involved in many proinflammatory intestinal pathologies, such as IBD. Activation of several PARs is also implicated in other disease-associated gut dysfunctions, including IBS, fibrosis and epithelial cell proliferation and cancer development. There have been several excellent, comprehensive reviews on these topics published in 2004 and 2005.^{30–33}

There are numerous ongoing studies aimed at elucidating the activities of PARs; however, the mechanisms that influence the abundance and distribution of the proteases that regulate PARs in the gastrointestinal tract and the downstream effects of their activation on mucosal function are relatively unknown. It is likely that proteases from multiple cell types, or potentially from luminal microbes, impact PAR signaling. It is also likely that the types of signaling response that are induced depend on the specific PAR that is activated, receptor location (i.e. on the apical vs basolateral side of the intestinal epithelium), interactions with other surface receptors, the duration of exposure to the protease, and the presence of regulatory and inhibitory factors. How these events impact gastrointestinal pathophysiology, specifically mucosal permeability, immune defense mechanisms, and pathogenic challenges, are important and largely unexplored areas of investigation, which will be the focus of the remainder of this Review.

HOST PROTEASES IN MUCOSAL BARRIER FUNCTION

The apical and basolateral surfaces of IECs are exposed repeatedly to an array of proteases that can, through one or more mechanisms, induce rapid changes in mucosal barrier function. Mucosal permeability depends, in part, on the regulation of the dynamic and complex intercellular tight junction that is linked structurally and functionally to the IEC actin cytoskeleton.

One mechanism by which proteases regulate the mucosal barrier function is illustrated by the PAR2-mediated increase in epithelial permeability after the injection of trypsin or PAR2-activating peptide into the intestinal lumen,³⁴ thereby coupling PAR2 activation with tight-junction modulation and the cytoskeleton.³⁵ Another example is the increase in tight junction permeability by zonulin, a serine protease homologue,^{36,37} with the consequent passage of environmental or luminal antigens across the intestinal epithelial barrier. Two key environmental stimuli result in intestinal zonulin release and activation of its pathway: bacterial contamination of the small intestine³⁸ and luminal exposure to gliadin.^{39,40} When exposed to luminal gliadin, intestinal biopsies from celiac disease patients who are in remission release zonulin, with subsequent binding of zonulin to the cell surface, rearrangement of the cell cytoskeleton, loss of occludin and ZO-1 protein-protein interactions, and increased intestinal permeability.^{40,41}

These results suggest that gliadin activates zonulin, leading to increased intestinal permeability to macromolecules. Because increased intestinal permeability and tight-junction dysfunction occur in various clinical conditions, including food allergies, infections of the gastrointestinal tract, autoimmune diseases and IBD,⁴² it is possible that zonulin and/or additional proteases or protease cascades contribute to these disease processes. Further studies are needed to evaluate the hypothesis that protease-induced compromise of tight-junction integrity with diminished gastrointestinal barrier function yields a deleterious immune response to luminal antigens, triggering diverse disease conditions.

THE INTERPLAY OF HOST AND ENTERIC PATHOGEN PROTEASES

Although enteric pathogens have or secrete various proteases, there are limited details available on specific enteric pathogen proteases and how these enzymes, along with host proteases, influence disease pathogenesis. From the host perspective, MMP-7, which is expressed by Paneth cells, colocalizes with antimicrobial peptides known as cryptdins, and MMP-7 is responsible for processing cryptdins to their active form.⁴³ Enteric pathogens stimulate the rapid release of cryptdins, and both *Escherichia coli* and *Salmonella typhimurium* survive in greater numbers and are more virulent in MMP-7-deficient mice, a finding that is consistent with the importance of MMP-7 and cryptdins to disease pathogenesis.⁴⁴ Similarly, stromelysin-1 (MMP-3)-deficient mice show delayed bacterial clearance and onset of mucosal CD4⁺ T-cell responses.⁴⁵

Citrobacter rodentium, a mouse pathogen related to enteropathogenic and enterohemorrhagic *E. coli*, stimulates the release of host trypsin-like serine proteases and granzyme A.⁴⁶ Diminution of the pathogenic effects of *C. rodentium* in PAR2-deficient mice provides support for the role of activated PAR2 in mediating inflammation in infectious colitis.⁴⁶ The clinical outcome of infectious colitis is probably modulated by other human neutrophil serine proteases, including granzyme B, that have antibacterial properties,⁴⁷ as well as macrophage-secreted proteases including MMPs.

Macrophage proteases are thought to be important because macrophages are most abundant in the gastrointestinal mucosa.⁴⁸ Classically activated macrophages induced by select proinflammatory T_H1 cytokines are key regulators of inflammatory responses to bacteria and antigens that breach the epithelial barrier, whereas alternatively activated macrophages induced by T_H2 cytokines are vital for the host defense against enteric nematodes.⁴⁹ Although specific details are lacking, these macrophage subsets might elaborate distinct protease profiles. Lastly, host proteases might also enhance the bioactivity of bacterial toxins, such as the enhancement of the cytotoxicity of Shiga toxin type 2d by elastase.⁵⁰ Disease manifestations might, therefore, in part, be dependent on the protease profile associated with specific host cells and immune responses to an enteric pathogen.

Only recently has it been appreciated that enteric pathogens use specific proteases to stimulate gut secretion or inflammation, or to inhibit host immunity and, therefore, promote disease. One example of this is the *Bacteroides fragilis* zinc-dependent metalloproteinase enterotoxin, which is the primary virulence factor of enterotoxigenic *B. fragilis* (ETBF). This enterotoxin stimulates the rapid release of colonic epithelial cell surface proteins, cleavage of E-cadherin and activation of colonic epithelial cell signal transduction *in vitro*, triggering a rapid reduction in the function of colonic epithelial cells and the human colonic barrier, colonic epithelial cell shape changes, the release of proinflammatory cytokines and colonic epithelial cell proliferation.⁵¹ These activities might contribute to ETBF-associated diarrheal disease, IBD, and colorectal cancer. Similarly, the secreted zinc-dependent hemagglutinin/protease of *Vibrio cholerae* has mucinase activity and contributes to enterotoxicity, possibly by stimulating cleavage of occludin, whereas the enterotoxin of *Clostridium perfringens* might cleave select claudins: occludin and claudin cleavage might reduce barrier function.⁵²

Serine protease autotransporters of *Enterobacteriaceae* (SPATEs) are a family of secreted proteases with divergent substrate specificities that contribute to intestinal disease.⁵³ Bacterial-secreted IgA₁ proteases that cleave the human immunoglobulin A₁ hinge region are thought to contribute to mucosal bacterial colonization.⁵⁴ Lastly, nonprotease enteric toxins (e.g. *Clostridium difficile* toxin B) initiate signaling cascades that involve one or more host proteases, contributing to the final gastrointestinal pathology.⁵⁵ Despite the progress made in identifying these protease-mediated interactions between pathogen and host,^{53–56} few specific mechanistic details are available.

Nematodes elaborate different proteases depending on their stage of development (e.g. egg, larva, or adult), proteases that contribute to their ability to migrate, and adapt to and survive gut host defenses. Nematode larvae secrete proteases with serine-protease-like and metalloproteinase-like activities⁵⁷ that are important for tissue penetration and ECM degradation. Infection by a number of different enteric nematodes results in an upregulation of PAR1 and PAR2 expression in the small intestine, coincident with altered responses to PAR-activating peptides.⁵⁸ These changes are dependent on the actions of IL-13 and its signaling molecule STAT6, and are, therefore, consistent with nematode proteases being immunomodulators. These proteases might also contribute to the increased intestinal permeability associated with nematode infection.^{59–61}

Nematodes also elaborate protease inhibitors that can modulate the activity of host proteases.⁶² Many nematodes elaborate cystatins, a group of cysteine protease inhibitors that contribute to immunosuppressive and anti-inflammatory effects during chronic infections.⁶³ For example, nippocystatin, which is secreted by *Nippostrongylus brasiliensis*, inhibits host antigen processing and antigen-specific immune responses.⁶⁴ Nematodes also produce serpins that are believed to protect the parasite from host digestive enzymes and contribute to the ability of that nematode to infect a specific host.⁶² By contrast, some nematode serine protease inhibitors might be antigenic, stimulating a protective host immune response against bacterial-induced T_H1 inflammation.⁶⁴

The mechanisms described above, combined with the fact that nematodes secrete antimicrobial peptides (e.g. cecropin 1) that have broad anti bacterial activity against enteric pathogens,⁶⁵ suggest that nematode and/or bacterial interactions in the intestine modulate the luminal environment and, hence, the contribution of bacteria to gastrointestinal inflammatory pathologies.⁴⁶ Together, these data suggest that pathogen-secreted proteases participate in the regulation of IEC function, the development of host immune responses and influence protease activation and secretion by the host.

PROTEASES IN GUT INFLAMMATION

Inflammation is one of the first responses of the intestine to irritation, injury, loss of mucosal integrity, and infection. Inflammation induces a number of effects on mucosal intestinal function that depend, in part, on the nature of the inflammatory infiltrate. An increased number of neutrophils and classically activated macrophages are dominant in the inflammatory pathologies that are dependent on bacteria, including IBD.⁶⁶

Infiltrating immune cells initiate a cascade of events, including the release of proinflammatory cytokines, nitric oxide, eicosanoids, and proteases into the intestinal mucosa, that contribute to both the initiation and maintenance of inflammation. Neutrophils recruited to the intestinal mucosa elaborate proteases such as neutrophil elastase, cathepsin G and neutrophil leucocyte proteinase 3 (also known as myeloblastin), which are stored in granules as active enzymes and not only target pathogenic microbes, but also trigger the activation of PAR2 on IECs.⁶⁷

Work published in 2006 has shown that inhibition of, or deficiencies in, tryptase,⁶⁸ neutrophil elastase,⁶⁹ or neutrophil-derived MMPs⁷⁰ significantly attenuate mucosal inflammation and injury in experimental models of colitis. Conversely, mice deficient in MMP-2 are highly susceptible to the development of colitis,⁷¹ which suggests that the relative activities of MMPs dictate the inflammatory outcome in colitis. Neutrophil proteases are not only important for the degradation of phagocytosed products, but also for extracellular proteolysis during the development of inflammatory pathologies. In addition, after being stimulated by proinflammatory cytokines (e.g. TNF, IL-1), IECs release proteases.⁴ Both infiltrating cells and IECs, therefore, might contribute to the increased levels of proteases, such as MMP-9 and the TNF-converting enzyme, ADAM 17 (also known as TACE), that are found in the inflammatory infiltrates of IBD patients.⁷²⁻⁷³ Cellular release of MMPs would degrade ECM components during inflammation, contributing to the loss of mucosal integrity, and facilitating both enhanced penetration of the mucosa by inflammatory cells and mucosal interactions with luminal antigens.

Stress-induced enteropathies, allergy, nematode infection and reperfusion injuries all promote the release of mast-cell proteases in the gut, which will, therefore, affect the functions of neighboring IECs. Similar to other recruited inflammatory cells, mast cells express PARs, and serine proteases (e.g. tryptase, chymase and granzyme B) released from granules contribute to the host response to microbes and can also have a significant effect on mucosal function.²¹ Mast-cell tryptase is reported to regulate paracellular permeability of cultured colonic IECs through activation of PAR2.¹⁰ Mast cells, along with several other inflammatory and/or immune cells, are also important for mucosal healing. Relapsing cycles of injury and repair, however, can result in excessive deposition of ECM and fibrosis, a feature of several chronic inflammatory pathologies, including IBD and chronic irradiation. These conditions are associated with upregulation of PAR2.²¹⁻⁷⁴ PAR2 orchestrates various responses through its interaction with multiple immune and inflammatory cells, effector cells such as epithelial and smooth muscle cells, and enteric nerves. These findings support the exploration of antitryptase therapy for the treatment of colitis.⁷⁵

TISSUE REMODELING AND WOUND REPAIR IN THE INTESTINAL EPITHELIUM

Proteases are crucial for the resolution of inflammation and wound repair.⁷⁶ Components of the ECM, such as collagen, gelatin, elastin, fibronectin, proteoglycans and vitronectin, undergo continual remodeling by proteases. An imbalance between proteases and their inhibitors has an important role in the pathophysiology of several inflammatory disorders.⁴⁶

When inflammation-induced protease activities cannot be controlled by inhibitors, excessive remodeling and ECM degradation induce the loss of epithelial cell–cell and cell–matrix junctions, leading to intestinal destruction, and further amplifies and disturbs intestinal immune responses.

Many proteases, including collagenase-1 (MMP-1), stromelysin-2 (MMP-10), and MMP-7, as well as various protease inhibitors, are upregulated by cytokines released during wound repair, and contribute to intestinal epithelial recovery *in vivo*.⁷⁷ Macrophage migration is essential for resolution of acute inflammation and for the initiation of adaptive immunity in the inflammatory environment. This migration depends on fibrin and is associated with the integrin MAC-1, tissue-type plasminogen activator and its inhibitor PAI-1, and the endocytic receptor LRP-1 (LDL receptor-related protein-1).⁷⁸ Tissue-type plasminogen activator and urokinase plasminogen activator convert plasminogen to plasmin, a serine protease that degrades fibrin and multiple ECM components. Plasmin activates MMPs to degrade collagen further and to degrade other ECM proteins, thereby diminishing cell substratum adhesion and enhancing cell migration. ECM remodeling and modification of angiogenic growth factors by proteases triggers further new blood vessel growth,⁷⁹ which is critical for wound repair and re-epithelialization. There is evidence for potent angiogenic activity in the mucosa of patients with IBD, indicating that the local microvasculature undergoes intense inflammation-dependent angiogenesis.⁸⁰

The mechanisms governing regeneration of gastrointestinal tissue are complex and involve numerous growth factors and cytokines that stimulate the proliferation and migration of IECs. HGF, a growth factor with homology to the serine protease family that is secreted by mesenchymal cells, is a key promoter of the differentiation, migration, and growth of IECs during epithelial repair via stimulation of the HGF receptor (the Met proto-oncogene tyrosine kinase).⁸¹ HGF is cleaved to its active form by HGF activator (HGFA), which is a serine protease that is itself synthesized in a precursor form and activated by the coagulation protease, thrombin, in injured mucosa. The Kunitz-type protease inhibitor 1 (HGFA inhibitor type 1; HAI-1) acts as a reversible inhibitor and reservoir for HGFA on the surface of epithelial cells.

Inflammatory cytokines can stimulate the shedding of HGFA–HAI-1 complexes from the IEC surface, whereby HGFA efficiently activates pericellular HGF in injured mucosa. Regeneration of injured intestinal mucosa is impaired in HGFA-deficient mice.⁸² Pro-HGF can also be activated by the serine protease matriptase, which is implicated in enterocyte migration and turnover.⁸³ Matriptase activates several protease zymogens, urokinase plasminogen activator,⁸⁴ prostasin (an IEC serine protease),⁸⁵ and MMP-3,⁸⁶ as well as PAR2 *in vitro*,⁸⁷ suggesting that it is involved in a complex protease signaling network associated with mucosal barrier function, inflammation and other intestinal pathologies.

CONCLUSIONS

Despite making significant progress in many aspects of our understanding and treatment of intestinal disorders, there remain significant gaps in our knowledge. Gastrointestinal proteases have been well studied with regard to their role in digestion; however, it is now clear that intestinal epithelial biology and function might be controlled by a sophisticated interplay between proteases and their inhibitors and receptors, which are expressed by diverse cells of the intestinal epithelium.

It is important that we now identify the proteases and protease inhibitors present in the intestinal mucosa under defined physiological conditions, with the understanding that an imbalance between proteases, protease receptors and protease inhibitors, or in the rates of

internalization and degradation of protease receptors and receptor complexes, is likely to impact greatly on the nature and severity of immune and inflammatory responses.

The hijacking or mimicry of these protease pathways by enteric pathogens and their contributions to the promotion of inflammation and immunity are other important areas for future investigation. Understanding the mechanisms by which proteases function and the ways they are regulated in the complex micro environment of the cells of the gastrointestinal tract is of fundamental importance if we are to achieve a wider understanding of gastrointestinal physiology and disease.

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Figure 1.

Protease pools relevant to intestinal mucosal pathophysiology. The intestinal epithelial microenvironment generated by the pool of endogenous and exogenous proteases is finely controlled. By controlling the intestinal epithelial microenvironment, appropriate physiological enterocyte function is maintained and pathological situations such as inflammation, injury, and tumorigenesis can be reacted to. A few representative examples of the many proteases present in the gut are shown. Abbreviations: APC, antigen-presenting cell; BFT, *Bacteroides fragilis* zinc-dependent metalloproteinase enterotoxin; DPP IV, dipeptidyl peptidase 4; ECM, extracellular matrix; IEC, intestinal epithelial cell; MMP, matrix metalloproteinase; T_H, T helper cell; T_{REG}, T regulatory cell.

Figure 2.

Examples of protease signaling and inter-receptor crosstalk on the cell membrane. (1) Protease zymogen cascades. Many proteases are synthesized as inactive precursors that are converted to their active form by limited proteolysis. For example, zymogen conversions involving the coagulation cascade result in cleavage and conversion of prothrombin to active thrombin at the cell surface. (2) Protease signalling by activation of PARs. Proteolytic cleavage of the extracellular amino-terminal domain of PARs unmasks a new amino terminus that binds to the body of the PAR itself and acts as a 'tethered ligand', inducing G-protein-coupled intracellular signaling responses. The PAR can be disabled by proteolytic cleavage downstream of the tethered ligand sequence. (3) There is crosstalk between GPCR signaling pathways and other cellular signaling pathways via ligand-dependent signal transactivation. GPCR signaling induces membrane metalloproteinase (ADAM) activation and proteolytic release of EGF-like ligands, inducing phosphorylation and signaling via the EGF-receptor tyrosine kinase.^{17,18} Abbreviations: ADAM, a disintegrin and metalloproteinase domain; EGF, epidermal growth factor; GPCR, G-protein-coupled receptor; L, ligand; P, phosphate; PAR, protease-activated receptor.