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TITLE: Proteolytic Regulation of the Intestinal Epithelial Barrier: Mechanisms and Interventions

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**Proteolytic Regulation of the Intestinal Epithelial Barrier: Mechanisms and Interventions**

Inflammatory bowel diseases are characterized by chronic, progressive and destructive inflammation of the gastrointestinal tract. The two main forms of inflammatory bowel diseases, Crohn’s disease and Ulcerative Colitis, currently affect over 1 million Americans including military personnel, and the incidence among aging veterans is rising. Compromised intestinal barrier function underlies much of the pathology associated with many inflammatory bowel diseases. Matriptase is a membrane-anchored serine protease encoded by the *Suppression of Tumorigenicity-14* (*ST14*) gene that is required for epithelial barrier homeostasis. Here, we are investigating matriptase dysregulation and its contribution to the pathogenesis of acute colitis using the *St14* hypomorphic mouse model of matriptase deficiency. The project uses a mouse model that is genetically deficient in matriptase and an experimental model of inflammatory colitis, to determine molecular processes by which matriptase protects barrier function in inflamed mucosa, and to define the mechanisms by which matriptase becomes decreased during inflammation associated with inflammatory bowel diseases. In the first year of this grant, our findings support a critical protective role for matriptase in restoring barrier function to injured intestinal mucosa, such that its down-regulation by IL-13 likely enhances excessive activation of the immune system.

**Subject Terms**

Inflammatory bowel disease, colitis, intestinal barrier permeability, leaky gut
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INTRODUCTION:

Inflammatory bowel diseases (IBD) are characterized by chronic, progressive and destructive inflammation of the gastrointestinal tract. The two main forms of IBD, Crohn’s disease and Ulcerative Colitis, currently affect over 1 million Americans including military personnel, and the incidence among aging veterans is rising. Compromised intestinal barrier function is believed to underlie much of the pathology of IBD. Matriptase is a membrane-anchored serine protease encoded by Suppression of Tumorigenicity-14 (ST14) that is required for epithelial barrier homeostasis. Here, we are investigating matriptase dysregulation and its contribution to the pathogenesis of acute colitis using the St14 hypomorphic mouse model of matriptase deficiency. Matriptase expression and regulation are being studied using the St14 hypomorphic mouse and matriptase dysregulation is being investigated by subjecting St14 hypomorphic and control mice to an experimental model of inflammatory colitis. The goal of the proposed studies is to determine the mechanisms by which matriptase protects gut barrier function in inflamed mucosa. The specific aims are (1) to determine the molecular mechanisms that mediate matriptase protection during experimental colitis by defining the effects on immune responses and epithelial cell regeneration and repair, (2) to define mechanisms responsible for down-regulation of matriptase in inflamed mucosa, and (3) to determine the contribution of matriptase loss to barrier dysfunction induced by cytokines associated with IBD pathogenesis.
BODY:

Research accomplishments associated with each task outlined in the SOW:

**TASK1:** Seek regulatory approval for use of animals which includes the IACUC, University of Maryland, and the USAMRMC ACURO. Prior approval will also be obtained prior to any modifications of the protocol (months 1-36).

The protocol used in the mouse studies over the last year were approved by the IACUC of the University of Maryland, and the USAMRMC ACURO (letter dated April 30, 2012).

**TASK2:** To determine the molecular mechanisms that mediate matriptase protection during mucosal inflammation in experimental colitis. (months 1-36).

**OBJECTIVE:** To define mechanisms by which the intestinal epithelial barrier is protected by matriptase during inflammatory colitis.

We hypothesize that the intrinsic defect in the intestinal barrier associated with the failure to form functional tight junctions in ST14 hypomorph mice (1; 2) prevents gut barrier repair and therefore perpetuates destructive intestinal inflammation associated with colitis and IBD, whereas normal matriptase levels in control mice during recovery enables essential barrier repair that promotes colitis resolution. Our experiments test this hypothesis utilizing a standard experimental mouse model of DSS-induced colitis. In this colitis model, the initiating trigger, DSS, causes injury to the epithelial layer provoking activation of an innate immune response to luminal contents and the induction of barrier repair processes. The experimental tasks are designed to investigate whether the increased permeability of ST14 hypomorph intestinal epithelium causes recurring priming of the immune system with increased immune cell infiltration and activation, and/or whether diminished matriptase increases epithelial cell destruction and/or interferes with mechanisms of epithelial cell regeneration and repair.

The specific tasks are:

(a) To induce colitis in groups of ST14 hypomorph and control littermate mice by administration of DSS in drinking water, and then replace with water only. Mice will be monitored for body weights, survival and clinical symptoms (months 1-36).

This task requires experimental St14 hypomorphic mice and control littermate mice obtained from our IACUC approved breeding protocol 0113002. During this past year, we have had some problems in obtaining sufficient numbers of experimental mice, which has delayed the experiments. This was in part due to unusually low breeding during the first 6 months, which was caused by construction of a building near the animal facility. The St14 hypomorphic strain in general is not as robust as normal mice, as they are smaller at birth and more fragile than their control littermates. The increased stress resulting from the noise of construction had an unanticipated and significant negative impact on the number of litters and litter sizes. Now that this building has been completed, the mice have returned to normal breeding and we have initiated studies addressing the goals of this task.

In the initial studies, colitis was induced in adult St14 hypomorphic mice and control littermate mice by administration of 2% DSS in the drinking water for 7 days (optimal conditions for inducing colitis with this batch of DSS). Control mice were given drinking water only. Animals were monitored and weighed daily. At sacrifice, colon lengths, spleen weights and body weights were recorded. Oral administration of DSS is injurious to intestinal epithelium and causes an acute colitis which mimics human IBD in many respects (3; 4). The St14 hypomorphic mice lost weight much more rapidly than their control littermates after DSS treatment (Figure 1A), and developed colitis evidenced by a modest reduction in colon length (Figure 1B) and substantially reduced colon weights indicative of a watery stool (Figure 1C). Determination of a clinical disease score (5) based on stool consistency, presence or absence of fecal blood (Sure-Vue Fecal Occult Blood Test) and changes in body weight revealed that all mice exposed to DSS developed comparable signs of an inflammatory colitis through day 7 (Figure 2). In contrast, symptoms of clinical disease were more severe and
persisted in St14 hypomorphic mice, with an average clinical disease score on day 7 of 8.5 for the St14 hypomorphic mice compared with 4 for the littermate control group (Figure 2). These data show that matriptase deficiency in mice enhances disease severity. During the course of this study, we noted that male mice appear more susceptible to colitis than female mice. The reason for this is not known but we will continue to monitor this as we proceed with these experiments.

(b) To sacrifice mice at various time points (3-10/group) during the course of the DSS protocol to evaluate molecular markers of acute inflammation in the subepithelial lamina propria, including quantity and nature of immune cell infiltration, and inflammatory cytokine production (months 1-18).

Spleen weights were increased in DSS treated mice, a marker of systemic inflammation (Figure 3). Inflammation associated with the intestinal mucosa was investigated by removing tissue segments from identical regions of the jejunum, small intestine, and the colons of littermate control and St14 hypomorphic mice for histological examination. Chronic inflammation is associated with immune cell infiltration and increased production of inflammatory cytokines in the lamina propria of IBD patients (6; 7). Initial analyses of H&E stained tissues of the distal colons for microscopic injury shows increased infiltrates in the mucosa and submucosa in both the St14 hypomorphic and littermate control genotypes after DSS treatment (Figure 4). There are substantially increased inflammatory infiltrates and increased epithelial sloughing in the colons of St14 hypomorphic mice compared with their corresponding control littermates (Figure 4, d vs e & f).

Preliminary investigation of cytokines and inflammatory mediators produced in response to DSS-induced injury vs. water alone showed that the baseline level of TNFα is relatively low in both strains. The littermate control mice display a substantial increase in TNFα after 7 days, whereas St14 hypomorphic mice do not display a substantial increase from baseline levels (Figure 5A). Baseline levels of IL-13 in St14 hypomorphic mice appear increased relative to their littermate controls, and this balance appears to change after DSS treatment (Figure 5B), although additional mice will need to be examined given the large error in this result. These data suggest that the enhanced permeability of the St14 hypomorphic mice leads to increased inflammation and possibly an altered cytokine profile associated with chronic, IBD-like colitis.
Figure 4. H&E stained sections of colonic tissue segments from 2 different St14 hypomorph and littermate control mice after 7 days on DSS, showing the persistence of inflammatory infiltrates in the proximal and distal colons of St14 hypomorphic mice compared with the well-defined colonic crypts, and a normal submucosa layer of water alone-treated mice.

Figure 5. Comparison of relative mRNA levels of (A) TNFα and (B) IL-13 in the distal colons of St14 hypomorph and littermate control mice at day 7, measured by qPCR analyses.

(c) To sacrifice mice at various time points (3-10/group) during the course of the DSS protocol to assess gut permeability by serum FITC-dextran concentration after gavage and by ex vivo TEER measurements on colonic mucosa mounted transwell chambers. (months 1-18)

A ‘leaky’ mucosal barrier due to decreased barrier function is thought to underlie persistent and destructive mucosal inflammation in IBD and other intestinal disorders (8; 9). St14 hypomorphic mice show enhanced intestinal barrier permeability as measured by a 20% decreased baseline TEER of the distal colons of St14 hypomorphic mice (1) and ~2-fold higher levels of serum FITC-dextran after gavage (Figure 6A). We are currently investigating the changes in barrier permeability during DSS treatment along the different parts of the intestines.

Figure 6. Matriptase deficiency increases intestinal permeability. (A) FITC-dextran (4 kDa; 500 mg/g body weight) was instilled by oral gavage, and the concentration of FITC-dextran in blood collected was measured after 24 hours. (B) Ex vivo intestinal permeability was measured in the small intestinal segments of St14 hypomorph and littermate control mice after DSS treatment or water only.
intestine by ex vivo TEER measurements on colonic mucosa mounted in transwell chambers. We find that the baseline jejunal TEER of littermate control mice decreases by ~30% after DSS treatment (Figure 6B). The low TEER of the St14 hypomorphs is not substantially reduced and is similar to the TEER measured for littermate control mice after the DSS treatment, highlighting a persistent permeability defect associated with St14 hypomorphic mice that is similar to DSS-treated control mice. These data support the notion that the defective epithelial barrier in St14 hypomorphic mice likely contributes to persistent inflammation following DSS insult.

**d** To sacrifice mice at various time points (3-10/group) during the course of the DSS protocol to evaluate bacterial translocation to lymph nodes, spleen and serum.

Bacterial translocation as a measure of barrier permeability will be evaluated by qPCR amplification of bacterial DNA extracted from mesenteric lymph nodes, spleens and colons. We are currently collecting these tissue specimens to perform this analysis.

**e** To sacrifice mice at various time points (3-10/group) during the course of the DSS protocol to assess differences in claudins and other junctional proteins that have been reported to form a “leaky” barrier in certain tissues between ST14 hypomorphic and control littermates. (months 1-18)

We have prepared tissue segments from identical regions of the jejunum, small intestine, and the colons of littermate control and St14 hypomorphic mice for analysis of protein and mRNA expression. Protein levels of claudin-2 have been reported to be elevated in jejunal tissues of St14 hypomorphic mice (1) and we observe elevated claudin-2 protein expression in St14 hypomorphs compared to littermate control mice (Figure 7A). Quantitative PCR analysis of claudin-2 mRNA expression indicates that mRNA levels are not substantially different (Figure 7B), indicating that regulation of claudin-2 in the mouse intestine may be largely post-transcriptional.

**f** To sacrifice mice at various time points (3-10/group) during the course of the DSS protocol to compare markers of epithelial regeneration in the colonic mucosa of ST14 hypomorphic with control mice, including staining of distal colonic segments for Ki-67, BrdU label incorporation, and epithelial apoptosis (months 6-24)

Epithelial apoptosis is an important component of intestinal mucosal barrier regulation. Pathologic changes can occur that lead to a breakdown of the intestinal barrier when apoptosis is altered. Initial investigation of apoptosis in intestinal tissues was performed by immunostaining for Poly ADP ribose polymerase (PARP) cleavage in protein lysates. The results suggest that substantial apoptosis occurs following DSS treatment, evidenced by the loss of full length PARP, and the appearance of degradation products which are a marker for apoptosis (4). We plan to investigate other cell markers and in situ cell death by TUNEL assay of collected cryostat sections.

Given the potential role of matriptase in promoting epithelial cell differentiation and turnover, we are also investigating epithelial regeneration in the colonic mucosa of DSS-treated ST14 hypomorphic mice by immunostaining of colonic segments for Ki-67, a nuclear protein preferentially expressed during active phases of the cell cycle and which is absent in resting cells (10). The preliminary results show strong Ki67 staining in the colonic crypts of both St14 hypomorphic and control mice, which is substantially reduced after DSS treatment (Figure 8). As a second approach to assess epithelial regeneration, we injected BrdU (50mg/kg) at 2 hours prior to sacrifice and immunostained intestinal tissue sections for BrdU positive cells as a marker of DNA synthesis (11; 12). BrdU staining showed similar positive staining in colonic crypts, with substantially less positive cells after DSS treatment (Figure 9). Crypts per mouse will be scored for Ki67 and BrdU-labeled cells, and their position recorded relative to the base of the crypt.
Figure 8. Representative images of Ki67 staining of colonic epithelium of St14 hypomorph and control mice after water or DSS treatment.

Figure 9. Representative images of BrdU staining of colonic epithelium of St14 hypomorph and control mice after water or DSS treatment.
TASK3: To define mechanisms responsible for down regulation of matriptase in inflamed mucosa. (months 12-36).

OBJECTIVE: To test the hypothesis that matriptase is down-regulated by cytokine-mediated inflammatory signaling pathways induced by immune system activation.

We and others have shown that loss of matriptase and protein expression disrupts barrier function (1; 2). Thus the experimental tasks are designed to investigate how and when matriptase is regulated in inflamed mucosa and during barrier recovery using the in vivo β-gal reporter gene trap under the control of the matriptase promoter in both heterozygous and hypomorphic ST14 mice.

Some preliminary studies have been accomplished in the first year. It is planned to focus on this task in Years 2 and 3 as proposed in the timeline.

The specific tasks are:
(a) To induce colitis in groups of 3-10 ST14 hypomorphic (-/GT) and control (+/GT) mice by administration of DSS for 5 days in drinking water and then replace with water only. Mice will be monitored for body weights and clinical symptoms (months 12-36).
(b) To identify the time-dependent changes in matriptase promoter driven gene expression by X-gal staining, combined with co-staining approaches for molecular marker and cytokine expression. (months 12-18)
(c) To analyze tissue specimens by qPCR for total RNA levels and matriptase protein expression (months 12-24).
(d) To correlate matriptase gene expression with cytokine profiles to identify effectors of matriptase down-regulation during inflammation. (months 12-24)
(e) To treat mice with antibiotics for a 4 week period prior to DSS challenge. (months 18-36)
(f) To establish whole colon cultures from -/GT mice and +/GT mice and assess viability and efficacy for ex vivo studies. (months 12-36)
(g) To investigate specific regulation of the matriptase gene activity by specific inflammatory cytokines and incorporating cytokine pathway inhibitors (months 12-36)

We have induced colitis with DSS in groups of ST14 hypomorphic (-/GT) and control (+/GT) mice as described in Task 2a and removed tissue segments from identical regions of the jejunum, small intestine, and the colons of littermate control and St14 hypomorphic mice. Intestinal tissues were briefly fixed in PFA before staining with a β-galactosidase staining kit (Roche) overnight at 37°C. The tissues were post-fixed in PFA, embedded in paraffin, sectioned and counterstained with nuclear fast red. Our preliminary experiment was negative for X-gal staining, suggesting that optimization of technical conditions will be required for β-gal detection in these tissues.

To complement this approach, we have initiated qPCR studies to investigate changes in matriptase mRNA expression in the absence and presence of disease in tissue segments. Preliminary results show that matriptase expression is down-regulated in the intestinal mucosa of control mice during DSS induced injury (Figure 10A). We recently showed that matriptase and prostasin constitute a common proteolytic pathway where matriptase is indirectly activated by prostasin and functions on the cell surface to regulate barrier formation (13). Interestingly, our initial experiments show that prostasin is similarly down-regulated during DSS exposure, mirroring the changes in matriptase and correlating with increased intestinal permeability (Figure 10B).

![Graph A](image1.png)  ![Graph B](image2.png)

**Figure 10.** (A) Matriptase and (B) Prostasin gene expression are down-regulated in intestinal mucosa of control mice during DSS induced injury.
TASK4: To test the hypothesis that matriptase loss, induced by IL-13 during inflammation, mediates the IL-13 mediated effects on increased permeability and delayed epithelial barrier repair via derangement of tight junction integrity, increasing expression or localization of claudin-2 or other TJ proteins and stimulation of epithelial cell apoptosis (months 1-18).

OBJECTIVE: To test the hypothesis that matriptase loss, induced by IL-13 during inflammation, mediates the observed IL-13 effects on increased permeability and delayed epithelial barrier repair.

This task aims to identify a possible mechanism by which the IL-13/STAT6 signaling pathway induces apoptosis and barrier disruption. IL-13 production and claudin-2 expression are both increased in human ulcerative colitis and Crohn’s disease (14; 15), and matriptase is decreased (1). We will investigate the hypothesis that IL-13 induced down-regulation of matriptase contributes in a significant way to IL-13 dependent barrier dysfunction.

The specific tasks are:

(a) To grow cultures of polarized Caco-2 and T84 cells on permeable transwell supports and treat with IL-13 for various times (months 1-3).

We have grown human Caco-2 and T84 cells on permeable filter supports monitoring barrier development until the formation of highly polarized monolayers (TEER > 1500 Ohms.cm²). Over the last year, we have encountered some problems with barrier formation and TEER development in Caco-2 cells, so we have focused the experimental studies on T84 monolayers in the first instance. Caco-2 monolayers are now growing normally and we will be continuing investigation of IL-13 responses in these cells.

(b) To monitor the integrity of the barrier by measurement of TEER and by the flux of 4kDa FITC conjugated dextran across monolayers (months 1-3)

To model the effect of IL-13 on colonic epithelium, polarized human colonic T84 monolayers were treated daily with 10ng/mL IL-13 for 6 days. IL-13 was found to disrupt the epithelial barrier as measured by TEER development (Figure 11A) and is associated with increased macromolecular permeability monitored by measurement of the flux of 4kDa FITC-conjugated dextran (Fluka) across the T84 monolayer (Figure 11B).

(c) To investigate the effects of the restoration of recombinant matriptase to cell monolayers and determine the effects on IL-13 induced barrier permeability, claudin-2 levels, apoptosis, STAT6 phosphorylation and restitution rate in vitro (months 3-12)

At various times after IL-13 treatment, lysates from parallel cultures were prepared for RNA extraction and analyzed for matriptase, prostasin and claudin-2 mRNA expression by qPCR. Matriptase gene expression was down regulated at all times in the presence of IL-13. Prostasin gene expression decreased within the first 24 hours but appeared to recover to normal levels by 6 days. Claudin-2 gene expression was increased over the same time course, indicative of increased barrier permeability. These data indicate that IL-13 produced during inflammatory processes associated with IBD may down-regulate the matriptase pathway and prevent matriptase-mediated restoration of intestinal epithelial barrier function.

Figure 11. Effect of IL-13 on T84 barrier formation. T84 cells were seeded at a sub-confluent density and allowed to differentiate forming a highly polarized monolayer associated with TEER development. Polarized monolayers were treated daily with IL-13 (starting at time 0) and TEER monitored daily for 6 days. (A) Plot shows the effect of the absence and presence of IL-13 on TEER over the 6 days. (B) Paracellular permeability of IL-13 treated cultures to the passage of 4kDa FITC-dextran was measured on day 5 after addition of IL-13. The apparent permeability (P app) of T84 cultures is significantly increased compared untreated cells.
At various times after IL-13 treatment, lysates from parallel cultures were analyzed for protein expression by immunoblot analyses. We found that IL-13 treatment decreases matriptase protein expression. Claudin-2 protein levels were increased as we have observed previously for Caco-2 monolayers (1), whereas claudin-1 levels are unaffected by IL-13 (Figure 13).

IL-13 mediates its activities by binding to its receptor, comprised of the IL-4 receptor \( \alpha \) (IL-4R\( \alpha \)) and the IL-13 receptor \( \alpha_{1} \) (IL-13R\( \alpha_{1} \)) subunits, which triggers a signaling cascade leading to the phosphorylation of STAT6 and down-stream gene regulation. Recently it was shown that ulcerative colitis is associated with increased colonic epithelial STAT6 phosphorylation, and STAT6 inhibition prevents IL-13-induced apoptosis and barrier disruption (16). Treatment of T84 monolayers resulted in a substantial increase phospho-STAT6 (Figure 13), which correlates with reduced matriptase expression and increased permeability of T84 monolayers.

Apoptosis was monitored by immunoblot after IL-13 treatment in T84 monolayers by investigation of PARP cleavage and activated caspase 3. The data do not show significant PARP cleavage but an increase in activated caspase 3 (Figure 13), which may suggest an increase in epithelial cell death. This will be further investigated by TUNEL assay of fixed sections and co-staining for specific apoptotic cell markers.

We are currently in the process of investigating the effects of restoration of recombinant matriptase on these IL-13 mediated effects. When 5nM recombinant matriptase was added to the basolateral side of T84 monolayers compromised by exposure to IL-13, there was a significant improvement in the development of TEER of the cytokine-perturbed epithelial monolayers (Figure 14).
(d) To transfect epithelial monolayers with pcDNA plasmid vectors encoding human matriptase and control constructs, generate and characterize stable cell lines, and determine the impact on IL-13 induced barrier permeability, claudin-2 levels, apoptosis, STAT6 phosphorylation and restitution rate in vitro (months 4-18).

As a positive test of this approach, SW480 cells (which do not express endogenous matriptase) were transfected with human matriptase cDNA in pcDNA3.1 and vector alone, the cells selected with G418, and then grown in 24 well plates. Monolayers were ‘wounded’ and cell migration was monitored for 72h. Ectopic expression of matriptase induced a 35% increase in transmigration across the SW480 wound (Figure 15). While epithelial cells can close gaps in the epithelium by migration of neighboring cells into the defect, IL-13 has been reported to reduce the rate of restitution by 30% (14). Studies are planned to investigate T84 restitution in the absence and presence of IL-13 in the presence or absence of matriptase.

**TASK5:** Perform data analysis and prepare technical reports (months 1-36).

We are continuing data analysis, preparation of technical reports and planning publication of the findings.
KEY RESEARCH ACCOMPLISHMENTS:

- Matriptase plays a critical role in epithelial barrier formation and assembly.
- Matriptase deficiency leads to persistent, severe inflammatory DSS-induced colitis.
- Persistent colitis in ST14 hypomorphic mice is associated with an inability to recover TEER.
- Matriptase is down-regulated in intestinal mucosa during experimental DSS-induced colitis.
- Matriptase is down-regulated by IL-13 implicated in the pathogenesis of IBD
- Preliminary data suggest that down-regulation of matriptase during inflammatory colitis prevents gut barrier repair and that restoration of matriptase may ameliorate destructive inflammation.

REPORTABLE OUTCOMES:
None in this first year of the award.

CONCLUSION:

Compromised intestinal barrier function is strongly associated with the pathogenesis of IBD. This project seeks to understand a critical new mechanism by which intestinal epithelial barrier function can be compromised during inflammatory colitis that occurs during IBD. The enhanced susceptibility of matriptase hypomorph mice to DSS-induced injury and inflammatory colitis combined with the in vitro assays of barrier function, suggest that matriptase could be an important contributor to inflammatory colitis and repair of injured intestinal mucosa. These data show that matriptase deficiency in mice enhances disease severity and preliminary studies suggest that the nature of inflammation may be altered by the presence and absence of matriptase. These data support the notion that the defective epithelial barrier in St14 hypomorphic mice likely leads to persistent inflammation following DSS insult. Our work in the next year will focus on further molecular characterization of changes induced by DSS colitis and repair processes in St14 hypomorphic and control mice (Task 2), the in vivo regulation of matriptase during inflammatory colitis (Task 3), and completing the studies on the mechanisms involved in IL13 regulation of experimental epithelial barriers and permeability with a focus on the role of matriptase (Task 4).

The novel ‘barrier forming’ function of Matriptase is unexpected and has revealed a major gap in our understanding of a mechanism that regulates barrier permeability and which is perturbed in inflamed mucosa during colitis. The outcomes of the proposed studies are likely to substantially reshape our current view of factors and mechanisms that regulate intestinal epithelial barrier function. This will enable future studies of this pathway, and its dysregulation in patients with barrier dysfunction as well as in asymptomatic healthy subjects at risk of developing disease. The possibilities for harnessing this knowledge in therapeutic strategies to restore the barrier function of the gut to prevent or treat disease is considerable, since matriptase belongs to a class of enzymes that are a major focus of attention for the pharmaceutical industry as potential drug targets, and as diagnostic and prognostic biomarkers.
REFERENCES:


