Differential expression and regulation of ADAM17 and TIMP3 in acute inflamed intestinal epithelia

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Crohn’s disease; intestinal epithelial cell; neutrophil

CROHN’S DISEASE (CD) is a chronic inflammatory bowel disorder of unknown etiology (22). Clinical, epidemiological, and molecular genetic studies have provided evidence that both genetic and environmental factors are involved in CD pathogenesis (28, 34). There are at least 10 susceptibility genes, of which NOD2/CARD15 is one of most important (34). The NOD2 gene encodes an intracellular bacterial sensor involved in host innate immunity, and three loss-of-function mutations (R702W, G908R, and 1007fsInsC) have been identified in a significant percentage of patients with CD (17). At a cellular level, CD is characterized by an exaggerated inflammatory response with massive infiltration of polymorphonuclear leukocytes (PMNL) and secretion of proinflammatory cytokines that cause mucosal damage, ulceration, and loss of intestinal function (28, 34). However, the consequences of the interaction between PMNL and intestinal epithelial cells (IEC) are still not fully understood (13, 19). Current therapies are aimed at reducing inflammation with neutralizing anti-tumor necrosis factor-α (TNF-α), a key proinflammatory cytokine (30). Despite many clinical benefits, such a strategy is expensive and associated with adverse events, and ~25–40% patients are refractory.

The TNF-α-converting enzyme ADAM17 (TACE/CD155q) has emerged as an alternative candidate in targeting TNF-α and thus in treatment of chronic inflammation in CD (8). Indeed, ADAM17 is a transmembrane metalloprotease that cleaves the TNF-α precursor to generate the soluble active form of TNF-α, a phenomenon termed “shedding” (3). Its inhibitor TIMP3 (tissue inhibitor of metalloproteinase-3) is a secreted protein that negatively controls the ADAM17 activity (1). As a result, a delicate balance between ADAM17 and TIMP3 expression may be important in timely regulation of the inflammatory response, tissue degradation, and remodeling.

Although critical, very little is known about the regulation of ADAM17/TIMP3 expression during acute inflammation and even less about their relationship to the CD activity, a prerequisite for a therapeutic strategy. It has been shown that ADAM17 is expressed in the human colonic mucosa and increased in human inflammatory bowel diseases (IBD) (6). Similarly, TIMP3 is upregulated in the inflamed mucosa of patients with IBD, in correlation with TNF-α production (22). Interestingly, TNF-α is able to upregulate ADAM17 expression in endothelial cells, whereas regulation in epithelial cells remains elusive to date (7). Of particular importance, we have recently shown, using an in vitro model, that transmigration of PMNL across epithelia, the primary hallmark of an acute CD phase, induces the secretion of TNF-α by an intestinal epithelial cell line (2). PMNL accumulation within the epithelial crypts and the intestinal lumen directly correlates with the clinical disease activity, epithelial injury, patient morbidity, and susceptibility to colorectal cancer (11). Yet the molecular and cellular consequences of the interaction of PMNL with IEC are incompletely understood and a better understanding of...
this process will probably open up new therapeutic avenues for CD (18).

The aim of this work was to gain new insights into the regulation of the expression of ADAM17 and TIMP3 in IEC during PMNL-IEC interaction. We show for the first time that the ADAM17 protein is rapidly and transiently upregulated during PMNL transepithelial migration. At a molecular level, we demonstrate that the local production of TNF-α by IEC is required in upregulating ADAM17. Interestingly, we also show in biopsy specimens that, independent of the patients’ genetic background (NOD2/CARD15 status), a high level of expression of ADAM17, but not of TIMP3, correlates with strong infiltration by PMNL and the activity of the disease.

MATERIALS AND METHODS

Epithelial cell culture. T84 cells (American Type Culture Collection, passages 65-90), a human colonic carcinoma cell line, were grown and maintained as a confluent monolayer as previously described (15, 18, 19). For experiments, monolayers were grown and maintained confluent on collagen-coated 5-cm² polycarbonate filters as previously described (18).

PMNL transmigration. Human PMNL were isolated from whole blood by using a gelatin-sedimentation technique. The number of PMNL that transmigrated into the lower reservoirs was assayed by quantification of the azurophil granule marker myeloperoxidase (MPO), as described previously (15). Where indicated, similar experiments were performed in the presence of an anti-TNF-α antibody (0.5 µg/ml; Pharmingen, BD Biosciences, San Diego, CA) added to the upper and lower reservoirs.

Western blotting of ADAM17 and TIMP3. ADAM17 and TIMP3 expression was analyzed by Western blotting before and after PMNL migration by using inverted T84 monolayers grown on 5-cm² filters exposed for various times to PMNL for transmigration experiments, as described previously (18). Where indicated, the T84 inverted monolayers were alternatively incubated with H2O2 (0.001%; Sigma), as described previously (18). Where indicated, the T84 inverted monolayers were exposed for various times to PMNL for transmigration experiments, as described previously (18). Where indicated, the T84 inverted monolayers were alternatively incubated with H2O2 (0.001%; Sigma), as described previously (18). Where indicated, the T84 inverted monolayers were alternatively incubated with H2O2 (0.001%; Sigma), as described previously (18). Where indicated, the T84 inverted monolayers were alternatively incubated with H2O2 (0.001%; Sigma), as described previously (18). 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Paris, France) as described previously (16). Briefly, tissue cores with a dimension of 0.6 mm from each specimen were punched and arrayed in triplicate on a recipient paraffin block. Each core was spaced 0.2 mm apart. Before immunostaining, a 4-μm section stained with hematoxylin and eosin was reviewed to confirm the presence of morphologically representative areas of the original lesions.

**Immunohistochemistry.** Immunohistochemical methods were performed on serial 4-μm deparaffinized TMA sections processed as described (14). The following antibodies were applied for 45 min: monoclonal mouse anti-ADAM17 (Chemicon; 1:500), anti-TIMP3 (Chemicon; 1:400), and anti-MPO (Chemicon; 1:400). After being rinsed with PBS, sections were incubated with peroxidase-labeled anti-mouse IgG (DAKO Envision System, DAKO, Carpinteria, CA) for 45 min. For measurement of histological disease activity, the scoring system for histological abnormalities in CD mucosal biopsy specimens was used (26). After immunostaining, slides were analyzed with an image-analysis workstation (Spot Browser version 7; Alphelys, Paris, France), as described previously (14).

**NOD2/CARD15 genotyping.** Genomic DNA was obtained from intestinal biopsies by use of an XYZ extraction kit (ABCD efficient furnisher). Patient and control subjects were genotyped for the Arg702Trp (SNP8), Gly908Arg (SNP12), and Leu1007fsinsC (SNP13) variants in the NOD2/CARD15 gene as described by Lesage et al. (21). The PCR primers used in this study have been described previously (21).

**ADAM17 enzyme activity.** Untreated and treated T84 monolayers and digestive biopsies were solubilized in a buffer containing 10 mM Tris pH 7.4, 1 mM MgCl2, 0.2 mM CaCl2, and the protease inhibitor cocktail described above. The recombinant ADAM17 protein was used as a positive control. ADAM17 activity was measured by using the Innogenzyme ADAM17 activity kit (Calbiochem) as described by the manufacturer.

**Data analysis.** Assays were compared via the Student’s t-test. Values are expressed as means ± SE of n number of experiments. The association of ADAM17 and TIMP3 expression with categorical clinicopathological features was made by χ2 analysis. Calculations

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**Fig. 1.** Polymorphonuclear leukocyte (PMNL) transepithelial migration upregulates ADAM17 expression at protein and mRNA levels. A: rate of PMNL transmigration across T84 cell monolayers at different times. Shown is an MPO assay indicating the total number of PMNL after migration induced by f-MLP (formyl-met-leu-phe) (10^-7). Results are means ± SE for 6–12 monolayers. B: ADAM17 expression was detected by Western blotting of whole cell lysates of T84 cells (top). The positions of the ADAM17 proform (110 kDa) and active form (85 kDa) are shown. After stripping, equal loading of protein was verified by reprobing the same blots with an anti-ERK antibody (middle). Quantification of ADAM17 on an immunoblot by densitometric scanning (bottom). *Significantly different (P < 0.01). C: ADAM17 mRNA expression was assayed by quantitative RT-PCR of T84 monolayers after different times of PMNL transmigration. *Significantly different (P < 0.01) from control. D: confocal microscopic analysis of confluent T84 cells stained with an anti-ADAM17 antibody.
and analyses were performed with SPSS 11.5 for Windows (SPSS, Chicago, IL) and, where appropriate, were two tailed. *P* values < 0.05 were considered statistically significant.

**RESULTS**

Transepithelial migration of PMNL induces rapid and transient upregulation of ADAM17 in human colonic epithelial T84 cells. In response to f-MLP (10⁻⁷ M), the number of transmigrated PMNL present in the lower reservoirs increased from 0 to 24 h (2 ± 0.5, 12.5 ± 2, 19 ± 2.2, 29.5 ± 2.4, 38.9 ± 2.5, and 42.4 ± 1.9 × 10⁴ PMNL cell equivalents per monolayer for controls and 0.5, 1, 2, 4 and 24 h of transmigration, respectively) (Fig. 1A). As shown in Fig. 1B, Western blot analysis demonstrated that PMNL transmigration induced a striking increase in ADAM17 expression in T84 cells during the first 2 h (~4-fold increase) that lasted, at this high steady-state level of expression, for 2 h before returning to the basal level at 24 h (Fig. 1B). Quantitative RT-PCR (qRT-PCR) analysis revealed that, during the same period, PMNL transmigration did not increase ADAM17 mRNA expression in T84 cells, suggesting no early transcriptional activation of the ADAM17 gene by PMNL transmigration (Fig. 1C). Nevertheless, an approximately twofold increase in ADAM17 mRNA in T84 cells was observed after 24 h of PMNL migration (Fig. 1C), indicating late gene regulation by PMNL transmigration. Consistent with the above kinetic profile, confocal microscopy confirmed fully these results since a strong signal for ADAM17 was detected both on the apical side and in the cytosol of T84 monolayers after 0.5 and 1 h of PMNL transmigration (Fig. 1D) and accumulated at a high level at 2 h. Expression was maintained at 4 h but decreased after 24 h (Fig. 1D). We verified that no significant increase in the CD11b mRNA level, a PMNL marker, was observed after PMNL transepithelial migration in washed T84 monolayers (data not shown). Together these findings revealed that PMNL transmigration increased transiently, and strongly, ADAM17 protein levels in T84 monolayers.

**Fig. 2.** Effects of PMNL incubation, H₂O₂, HBSS without calcium [HBSS(−)], TNF-α, and anti-TNF-α treatment on ADAM17 protein levels of T84 monolayers. The ADAM17 protein was detected in T84 cell extracts by Western blotting of control cells (0) and after incubation with PMNL for 0.5, 1, 2, and 4 h without f-MLP (Aa), treatment with 100 μM of H₂O₂ (Ab), incubation with HBSS(−) (Ac), and treatment with 10 μM of TNF-α (Ba) and with an anti-TNF-α antibody during PMNL transepithelial migration (Bb). Arrows indicate the positions of the 110-kDa proform and the 85-kDa active form of ADAM17. The loading control for total protein was assayed by Western blotting using an anti-ERK total antibody (Bc). C: quantification of 3 independent experiments (means ± SE).
T84 cells mainly through a transcription-independent mechanism.

PMNL-induced ADAM17 upregulation in T84 monolayers is mediated via TNF-α but not via incubation with PMNL, H2O2, and tight junction opening. To promote PMNL adhesion onto T84 cells, but not migration, PMNL were incubated with T84 monolayers without f-MLP. ADAM17 protein levels were not increased in T84 cells by contact with PMNL, compared with control T84 cells (Fig. 2Aa). Similarly, T84 monolayers incubated for different periods with H2O2 did not show any modulation in ADAM17 levels compared with untreated monolayers (Fig. 2Ab). Finally, opening of tight junctions with HBSS without calcium did not influence ADAM17 protein levels in T84 epithelial cells. As shown in Fig. 2Ac, incubation of T84 cells with recombinant TNF-α was sufficient to markedly increase the expression level of ADAM17 at 2 and 4 h, reminiscent of the kinetic profile observed for PMNL-induced effects. Quantification of three independent experiments confirmed this increase in ADAM17 after a 2- and 4-h incubation with TNF-α. To show that upregulation of ADAM17 in the intestinal epithelium during PMNL transepithelial migration may be caused by TNF-α, we performed PMNL transepithelial migration in the presence of an excess of neutralizing anti-TNF-α antibodies in the upper and the lower reservoirs. As shown in Fig. 2Bb, the presence of anti-TNF-α antibodies prevented most of the PMNL-induced upregulation of ADAM17, underscoring the key role played by TNF-α in ADAM17 upregulation during the PMNL transepithelial migration process. The loading control for total protein was assayed by Western blotting using an anti-ERK total antibody (Fig. 2Bc). Finally, quantification of three independent experiments confirmed increased ADAM17 only in T84 cells incubated in TNF-α for 2 and 4 h (Fig. 2C).

PMNL transepithelial migration induces a late increase in the TIMP3 protein level in epithelial T84 cells. PMNL transmigration did not modify the TIMP3 protein level at 0.5, 1, 2,
and 4 h but significantly increased it at 24 h (Fig. 3A). Using qRT-PCR, we consistently and concurrently showed that only prolonged PMNL transmigration for 24 h promoted an approximately twofold increase in TIMP3 mRNA in T84 cells (Fig. 3B). These observations were confirmed by immunofluorescence since the TIMP3 protein was barely expressed on the apical side of control T84 monolayers (Fig. 3C) and after 0.5, 1, 2, and 4 h of PMNL transmigration before showing a strong increase at 24 h (Fig. 3C).

TIMP3 expression in T84 cell monolayers is modulated by TNF-α but not by PMNL contact, H2O2, and HBSS(−) treatment. The TIMP3 protein level was not modified by incubation with PMNL compared with control T84 cells (Fig. 4Aa). In addition, incubation of T84 cells with H2O2 failed to affect the TIMP3 protein level (Fig. 4Ab), as observed for the opening of tight junctions by addition of HBSS(−) (Fig. 4Ac). As shown in Fig. 4Ba, incubation of T84 cells with TNF-α increased significantly the expression level of TIMP3 at 24 h. This was strengthened by the findings that TIMP3 upregulation by PMNL transmigration at 24 h was completely abrogated by an anti-TNF-α antibody, indicating that PMNL-induced TIMP3 upregulation was totally dependent on this proinflammatory cytokine (Fig. 4Bb). The loading control for total protein was assayed by Western blotting using an anti-ERK total antibody (Fig. 4Bc). Quantification of three independent experiments confirmed increased TIMP3 in T84 cells at 24 h of incubation with TNF-α (Fig. 4C).

ADAM17 protein expression is strongly increased in the inflamed intestinal epithelium of patients with CD. Intestinal biopsies were processed for ADAM17 immunostaining and mRNA quantification (Fig. 5, A–C). In patients with CD, the ADAM17 expression level was compared with the NOD2/CARD15 status (Fig. 5, B and C). Immunohistochemistry showed strong intracytoplasmic staining of ADAM17 in the intestinal epithelium that was markedly associated with PMNL infiltrates (Fig. 5A), which is in contrast to control biopsies that showed very weak ADAM17 expression (Fig. 5A). The presence of strong epithelial ADAM17 staining was accompanied by a very significant increase in the number of PMNL. The ADAM17 expression levels in the noninflamed colon and

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**Fig. 4.** Effects of PMNL incubation, H2O2, HBSS(−), TNF-α and anti-TNF-α treatment on TIMP3 protein levels in T84 cell monolayers. TIMP3 protein immunoreactivity was detected in T84 cell extracts by Western blotting in control cells (0) and in cells after 0.5, 1, 2, and 4 h of contact with PMNL (Aa), treatment with 100 μM of H2O2 (Ab), incubation with HBSS without calcium [HBSS(−)] (Ac), treatment with 10 μM of TNF-α (Ba) and with an anti-TNF-α antibody during PMNL transepithelial migration (Bb). The loading control for total protein was assayed by Western blotting using an anti-ERK total antibody (Bc). C: quantification of 3 independent experiments (means ± SE).
ileum were moderately increased compared with healthy controls (Fig. 5, A and B). As shown in Fig. 5B, no differences in the ADAM17 staining level were noted in association with the NOD2/CARD15 mutation. Figure 5C shows that in patients with acute-phase CD the ADAM17 mRNA levels in the inflamed colon and in the inflamed ileum were slightly increased compared with healthy controls. In biopsies taken from CD patients with quiescent disease, the ADAM17 mRNA level was highly increased compared with healthy controls (Fig. 5C). As shown in Fig. 5C no differences in the level of ADAM17 mRNA were noted in association with the NOD2/CARD15 mutation.

**TIMP3 expression is not increased in the intestinal epithelium of CD patients during the acute phase.** In contrast to ADAM17, immunostaining showed that the TIMP3 level did not correlate with the number of PMNL, both in the ileum and
the colon (Fig. 6A). The TIMP3 expression levels in the inflamed colon and ileum were not increased compared with healthy controls (Fig. 6, A and B). Immunohistochemistry showed stronger staining for TIMP3 in the intestinal epithelium of patients in a quiescent phase of CD (Fig. 6A), compared with control biopsies (Fig. 6A). Intracytoplasmic staining of the intestinal epithelium was continuous in the ileum and the colon. Besides intestinal epithelial cells, scattered cells stained positive for TIMP3 in the lamina propria. As shown in Fig. 6B, no difference in the level of TIMP3 staining was noted according to the NOD2/CARD15 status. TIMP3 mRNA levels were constant throughout the ileum and the colon (data not shown). Figure 6C shows that in patients with acute-phase CD, the TIMP3 mRNA levels in the inflamed colon and in the inflamed ileum were not significantly different compared with healthy controls, with a trend toward a slight decrease. In biopsies

![Image](https://example.com/image.png)

**Fig. 6.** TIMP3 expression in intestinal epithelium of CD patients. A: immunohistochemical staining of TMAs from colon and ileum biopsies of control subjects, patients in an acute inflamed phase of CD, and patients in a quiescent noninflamed phase of CD. Each spot shows representative tissue for TIMP3 immunostaining at a low original magnification (×100) (left) and at a high original magnification (×400) (right). B: positive cell density from colon and ileum biopsies in control subjects, patients presenting with an acute inflamed phase of CD with or without a NOD2/CARD15 mutation, and patients presenting with a quiescent noninflamed phase of CD with or without a NOD2/CARD15 mutation, for TIMP3, *P < 0.01. C: TIMP3 mRNA levels from colon and ileum biopsies in control subjects, patients presenting with an acute inflamed phase of CD with or without a NOD2/CARD15 mutation, and patients presenting with a quiescent noninflamed phase of CD with or without a NOD2/CARD15 mutation, *P < 0.01 for the group assessed by real-time RT-PCR.
taken from CD patients with quiescent disease, the TIMP3 mRNA level was increased compared with that in healthy controls (Fig. 6C). As shown in Fig. 6C, no differences in the level of TIMP3 mRNA were noted in association with the NOD2/CARD15 mutation.

High expression of the ADAM17 protein in patients with CD is correlated with the intensity of mucosal PMNL infiltrates and with the number of IEC-associated PMNL. A high level of PMNL infiltration was observed in patients with acute CD, whereas this level was low in patients in a quiescent phase. The activity of colitis (PMNL score) was graded as mild (1), moderate (2), intense (3), or severe (4) according to MPO staining (Fig. 7A). ADAM17 staining exhibited a significant correlation to neutrophil epithelium infiltration (Fig. 7B). ADAM17 staining was faint in biopsies showing a low score for PMNL infiltration and was strong in biopsies with a high score for PMNL (Fig. 7B). Conversely, TIMP3 staining did not correlate with the score for PMNL in digestive biopsies (Fig. 7C).

ADAM17 activity is increased in IEC in response to PMNL transepithelial migration and in digestive biopsies from patients in an acute phase of CD. ADAM17 enzyme activity of T84 cells was measured at different times during PMNL transepithelial migration by use of a dinitrophenol-labeled peptide mimicking the ADAM17 cleavage site of pro-TNF-α. As shown in Fig. 8A, we observed that PMNL transmigration induced a strong, rapid, and transient increase in the ADAM17 activity in T84 cells at 2 and 4 h that decreased at 24 h of PMNL transepithelial migration, which is in agreement with the above-observed kinetics of ADAM17 protein expression. Interestingly, we observed that the ADAM17 activity from ileum and colon biopsies was higher from inflamed tissues taken in the acute phase of CD than from tissues taken in the quiescent phase of CD or in healthy control subjects (Fig. 8B). Additionally, the ADAM17 activity correlated with greater severity of CD evaluated histologically in fresh biopsies and assessed using the scoring system described by D’Haens et al. (9) (Fig. 8C). The ADAM17 activity was faint in biopsies with lesser histological severity (Fig. 8C).

**DISCUSSION**

In this study we show that the early phase of acute inflammation is associated with a strong increase in expression of ADAM17, but not of TIMP3 in IEC. This finding was obtained with both an in vitro cell model system and a large series of

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**Fig. 7.** ADAM17 immunostaining correlates with the intensity of mucosal PMNL infiltration, whereas TIMP3 staining is not linked to PMNL infiltration. A: scores for PMNL according to myeloperoxidase staining: mild (1), moderate (2), intense (3), and severe (4) (anti-MPO antibody, peroxidase ×100). B: correlation between ADAM17 immunostaining and score for PMNL. C: absence of correlation between TIMP3 immunostaining and score for PMNL.
tissue specimens from patients with CD. Moreover, we demonstrate that this early upregulation of ADAM17 was linked to TNF-α production during PMNL transepithelial migration. A strict parallel between the results obtained in vitro using the T84 model (where only two cell components, i.e., the intestinal epithelial cells and the PMNL are present) and those obtained from mucosa of patients with CD (where in addition to these cell components, other components such as endothelial cells, mononuclear inflammatory cells, and fibroblasts are present) is difficult and limiting. However, despite the imperfections of the T84 in vitro model, it is a powerful approach that allows examination of the consequences of transepithelial migration of PMNL across an epithelial intestinal barrier and of the molecular biology of the neutrophil-epithelial cell interaction (19). Moreover, we have focused the ex vivo study of patient tissues on mainly the correlation of the intensity of the PMNL infiltrate and ADAM17 staining.

We showed in vitro that ADAM17 was upregulated at the protein level in T84 cells during PMNL transepithelial migration. We demonstrated that the upregulation of ADAM17 protein in IEC was not modified by free radical oxygen exposure.

The neutralization of TNF-α with specific antibodies is accompanied by a remarkable clinical response in patients with IBD (29). Pro-TNF-α is initially produced as a membrane-bound form that is processed by ADAM17 (4). We demonstrated previously that PMNL transepithelial migration across IEC monolayers induced TNF-α production (2). Moreover,
TIMP3 controls TNF levels by inhibiting ADAM17 in vivo and thereby prevents spontaneous inflammation (25). Finally, the effect of TNF-α on ADAM17 expression has been poorly investigated to date. This prompted us to investigate whether the local production of TNF-α by IEC could regulate the expression of ADAM17 and TIMP3. We provide evidence that TNF-α is necessary and sufficient to timely regulate the expression of both partners, with kinetics reminiscent of those observed in response to PMNL transmigration. On the basis of the above observation, we propose a model in which during acute inflammation PMNL transmigration induces the expression of TNF-α by IEC that would first upregulate the expression and the activity of ADAM17, further amplifying the local production of TNF-α. Thereafter, TNF-α would downregulate ADAM17 and concurrently increase the expression of TIMP3, events that would both decrease the ADAM17 activity. Previous reports have shown a high ADAM17 activity in colonic biopsies obtained from patients with IBD (6). However, a discrepancy in the results has been obtained for CD samples (6). Our ex vivo studies showed that ADAM17 was mainly overexpressed during the acute phase of CD, whereas TIMP3 was upregulated during the quiescent phase of CD. These findings shed light on the pathophysiological relevance of the ADAM17/TIMP3 balance in CD.

It has been suggested that the presence of a mutation in the NOD2/CARD15 gene may influence the phenotype of the disease (10). In this study we show that variations in the expression of ADAM17 and TIMP3 in the colon and ileum of patients with CD was independent of the NOD2/CARD15 status. Apart from influencing the disease itself, the host’s genetic background may also have potential implications for therapy. In this regard, we did not find any correlation between the NOD2/CARD15 status and clinical response in refractory patients to steroids and/or azathioprine, treated with anti-TNF-α therapy. These results are in agreement with previous studies showing the absence of association between the three common mutations of NOD2/CARD15 and a short- or long-term response to infliximab (24, 31).

Finally, we demonstrate that the ADAM17 activity was strongly increased in vitro in the early phase of PMNL transmigration and ex vivo in CD in an active phase, characterized both by a high score of PMNL and a high histopathological score of activity according to D’Haens et al. (9). However, we cannot totally exclude in this study that the ADAM17 present on human PMNL stimulated by formyl peptides or by other modulators may participate in the increase in the ADAM17 activity (32).

Although Wei et al. (33) showed that the inhibition of the extracellular region of ADAM17 by the inhibitory domain of TIMP3 results in positive cooperativity, our study demonstrates that ADAM17 and TIMP3 can be differentially regulated in intestinal epithelial cells during PMNL transepithelial migration. Further characterization of such PMNL-stimulated effects on epithelial cells will improve our understanding of the role of migrating leukocytes in epithelial function. From the perspective of new therapeutic options, our findings collectively point to the importance of broadening and deepening our understanding of the ADAM17 and TIMP3 status in CD.

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REFERENCES

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