

Secretory IgA mediates retrotranscytosis of intact gliadin peptides via the transferrin receptor in celiac disease

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Celiac disease (CD) is an enteropathy resulting from an abnormal immune response to gluten-derived peptides in genetically susceptible individuals. This immune response is initiated by intestinal transport of intact peptide 31–49 (p31–49) and 33-mer gliadin peptides through an unknown mechanism. We show that the transferrin receptor CD71 is responsible for apical to basal retrotranscytosis of gliadin peptides, a process during which p31–49 and 33-mer peptides are protected from degradation. In patients with active CD, CD71 is overexpressed in the intestinal epithelium and colocalizes with immunoglobulin (Ig) A. Intestinal transport of intact p31–49 and 33-mer peptides was blocked by polymeric and secretory IgA (SIgA) and by soluble CD71 receptors, pointing to a role of SIgA–gliadin complexes in this abnormal intestinal transport. This retrotranscytosis of SIgA–gliadin complexes may promote the entry of harmful gliadin peptides into the intestinal mucosa, thereby triggering an immune response and perpetuating intestinal inflammation. Our findings strongly implicate CD71 in the pathogenesis of CD.

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Abbreviations used: CD, celiac disease; dIg, dimeric Ig; mIg, monomeric Ig; PEG, polyethylene glycol; pIg, polymeric Ig; RP-HPLC, reversed-phase HPLC; SC, secretory component; SIg, secretory Ig; TAMRA, tetramethyl-6-carboxyrhodamine; Tf, transferrin; Tgase, transglutaminase.

Celiac disease (CD) is an inflammatory enteropathy induced by gluten-derived prolamines in genetically susceptible individuals. CD affects about 1 in 100 individuals in Europe and the United States (1, 2). The associated intestinal inflammation results from synergism between innate and adaptive immune responses to gliadin peptides. The adaptive immune response is orchestrated by CD4⁺ T cells recognizing various deamidated gliadin peptides (3), including a

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33-mer (peptide 56–88 [p56–88]) (4), bound to HLA-DQ2/8 molecules (5). p56–88 is a powerful immunodominant gliadin peptide extremely resistant to gastrointestinal digestion and has far higher T cell stimulatory potency than its 12-mer counterparts (6). The innate immune response in CD is triggered by a distinct set of gliadin peptides: one prototype innate peptide is p31–49, common to the N terminus of A-gliadins and shown to be toxic for CD patients both in vitro and in vivo (7–9). This peptide was recently shown to stimulate the synthesis of IL-15 (10, 11), a proinflammatory cytokine that can promote the

CD4⁺ adaptive immune response (11) and activate cytotoxic activity and IFN- γ production in intraepithelial lymphocytes (12–14).

This activation of the local immune system implies that undigested gliadin fragments present in the intestinal lumen somehow cross the intestinal epithelium. Indeed, apical to basal transport and processing of gliadin peptides, particularly of p31–49 and 33–mer, are severely altered in active CD, leading to the release of intact peptides on the basal side, whereas the same peptides are almost entirely degraded during their intestinal transport in control individuals and treated CD patients (15, 16). Several lines of evidence argue against simple nonspecific paracellular leakage of gliadin peptides across the celiac mucosa. In particular, a 12–mer gliadin peptide, p57–68, is completely degraded during intestinal transport in patients with active CD, suggesting no paracellular leakage of molecules of this size or larger. In addition, only 0.3% of the apical peptide crosses the epithelium during a 3-h incubation period, arguing against free diffusion across a damaged mucosa. The “protected” transport of p31–49 thus involves a transcellular pathway (15, 16), which enables the peptide to escape lysosomal degradation. As active CD is associated with high concentrations of antigliadin IgA antibodies in the intestinal lumen (17, 18), we postulated that the transport of intact gliadin peptides might result from abnormal retrotranscytosis of IgA–gliadin complexes. Indeed, although antigliadin IgA antibodies are a hallmark of CD, they have no known pathogenic role. We obtained evidence that polymeric/secretory IgA (pIgA/SIgA) can mediate protected transport of p31–49 and 33–mer gliadin peptides through their binding to CD71, the transferrin (Tf) receptor. Importantly, we found that this receptor was abnormally expressed at the apical pole of enterocytes in patients with active CD. Although initially implicated in endocytosis of iron-loaded Tf, CD71 was recently recognized as an IgA receptor mediating mesangial deposition of IgA1 complexes in IgA nephropathy (19).

RESULTS

High molecular weight immune complexes containing gliadin-specific IgA and transglutaminase (Tgase) are present in patients with active CD

Active CD is associated with enhanced intestinal secretory immunity (17, 20) and with higher antigliadin antibody titers in serum and jejunal secretions than in controls and treated CD patients (17, 20, 21). In addition, gliadin colocalizes with Tgase (the autoantigen in CD) in the epithelium and subepithelium of patients with active CD (22). We postulated that, as in IgA nephropathy, high molecular weight immune complexes (23) might be present in CD patients. As shown in Fig. 1 A, antigliadin IgA antibodies and Tgase were detected within high molecular weight immune complexes, which were found in larger amounts in the serum and duodenal secretions of patients with active CD than in those of treated CD patients and controls. The presence of Tgase in these immune complexes may be related to the cross-linking capacity of this enzyme, contributing to the formation of IgA–gliadin–Tgase complexes.

Consistent with a possible role of gliadin-specific IgA in the luminal uptake of gliadin peptides in active CD, immunofluorescence studies of duodenal biopsies revealed large amounts of IgA at the apical pole of the surface epithelium in patients with active CD, whereas in control subjects and treated CD patients, IgA was restricted to the crypts and was not observed in villous epithelium (Fig. 1 B and Fig. S1, available at <http://www.jem.org/cgi/content/full/jem.20071204/DC1>). In patients with active CD, colocalization of IgA with cytokeratin or alkaline phosphatase (a brush-border enzyme) confirmed the presence of IgA at the apex of surface epithelial cells (Fig. 1 C).

CD71 is overexpressed in the apical pole of enterocytes in active CD

IgA involvement in the intestinal transport of gliadin peptides would imply the presence of IgA receptors on the apical pole of enterocytes. In addition to the pIg receptor (pIgR; also called membrane secretory component [SC]), which permits transcytosis of dimeric IgA (dIgA) from the lamina propria and its delivery into the intestinal lumen, complexed to the cleaved extracellular part of pIgR (bound SC) in the form of SIgA, enterocytes express a second receptor able to bind IgA, namely CD71 (19, 24). In normal intestinal epithelium, CD71 mediates the rapid endocytosis/recycling of Tf necessary to deliver iron to rapidly proliferating epithelial cells (25). The level of CD71 expression is also linked to body iron stores and correlates negatively with the serum iron concentration. CD71 can be up-regulated in CD patients with iron deficiency anemia (26). The recent demonstration that CD71 can bind IgA1 at the surface of mesangial cells in IgA nephropathy (19), together with a previous report indicating up-regulation of intestinal CD71 by IL-15 in CD (27), led us to investigate whether CD71 mediates abnormal IgA retrotransport in CD.

The distribution of CD71 in active CD was assessed in duodenal biopsies by both immunoperoxidase and immunofluorescence detection (Fig. 2, A and B). In control biopsies, CD71 expression was confined to the basolateral pole of villous epithelial cells and was observed at both the basal and apical poles of crypt epithelial cells. In contrast, in active CD, CD71 was strongly expressed all over the epithelial layer, including the flat surface epithelium. Staining was not restricted to the basolateral compartment but was also detected at the apical pole of epithelial cells in a subapical rather than strictly apical location, possibly corresponding to the apical recycling compartment from which this receptor is rapidly recycled to the cell surface (28). A similar abnormal distribution of CD71 expression was observed in patients with refractory celiac sprue who have a flattened overproliferating mucosa. In contrast, CD71 expression was normal in treated CD patients who had recovered a normal or subnormal epithelial architecture. The strong expression of CD71 contrasted with the lack of significant expression of CD89, the myeloid IgA Fc receptor (29), on either enterocytes or immune cells in the lamina propria of controls and CD patients (unpublished data) (30).

Collectively, these results were compatible with a role of CD71 in IgA binding at the apical surface of enterocytes in active CD.

IgA colocalizes with CD71 and p31-49 at the apical pole of enterocytes in active CD

The possible role of apically expressed CD71 in the binding of IgA–gliadin peptide complexes in patients with active CD was first addressed by performing colocalization immunofluorescence studies on frozen sections. Colocalization (white) of IgA (green) and CD71 (blue) was observed at the apical pole (and the basal pole) of epithelial cells (Fig. 2 C) in fresh biop-

sies from patients with active CD but not from treated CD patients or controls. The presence of CD71 at the brush-border membrane of enterocytes in patients with active CD and its colocalization with IgA was confirmed by immunogold electron microscopy (Fig. 2 D and Fig. S2, available at <http://www.jem.org/cgi/content/full/jem.20071204/DC1>).

As SIgA is the main IgA subtype present in the intestinal lumen, we examined whether it can bind CD71. The capacity of pIgA and SIgA to bind to Daudi cells, which express CD71 as the only IgA receptor (19), was studied by flow cytometry. Both pIgA and SIgA were able to bind Daudi cells, and this

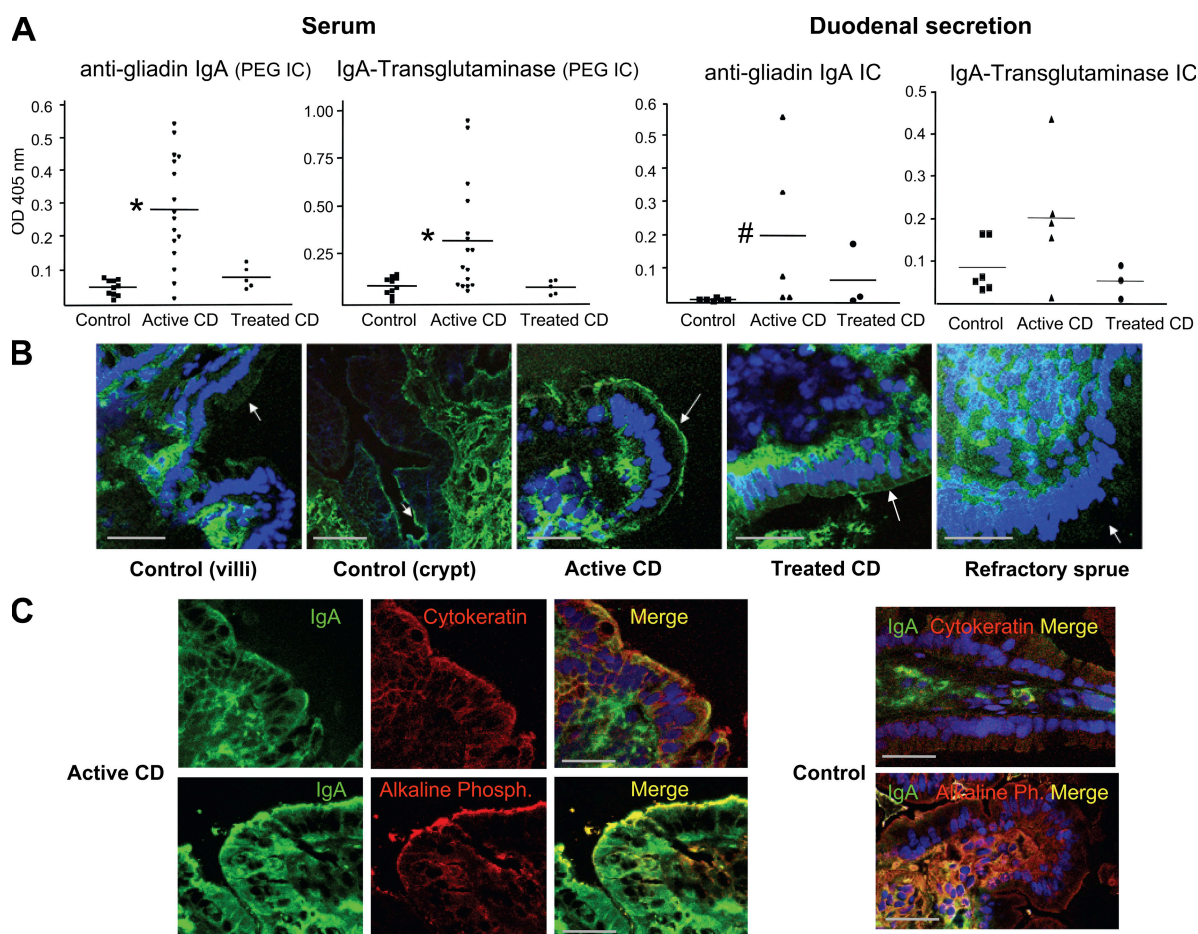


Figure 1. IgA and IgA–gliadin complexes are overexpressed in CD. (A) Analysis of IgA immune complexes (IC) isolated from the serum and duodenal secretions of patients with active or treated CD and controls. PEG precipitates containing high molecular weight IC were analyzed by ELISA. Plates were coated with Frazer's fraction (pepsin/trypsin gliadin hydrolysate) or anti-Tgase antibody, followed by anti-human IgA–horseradish peroxidase. Results are presented as OD values obtained with all sera or duodenal secretions tested. A significant increase in IC recognizing gliadin peptides and containing Tgase was observed in the active CD group compared with the treated CD and control groups in both serum and duodenal secretion. Horizontal lines represent medians. *, $P < 0.0005$ and 0.01 compared with the control and treated CD groups, respectively; #, $P < 0.01$ and 0.02 compared with the control and treated CD groups, respectively. (B) IgA is concentrated at the apical pole of surface enterocytes in active CD. Cryosections of duodenal mucosa from control subjects, patients with active or treated CD, and patients with refractory celiac sprue were labeled with anti-IgA–FITC antibody and TOPRO-3 (blue nuclei). In controls, treated CD patients, and patients with refractory celiac sprue, IgA staining of epithelial cells was located at the basal pole of villous cells and at the apical and basal poles of crypt cells. In contrast, in patients with active CD, IgA staining was concentrated at the apical pole of the surface epithelium (and of crypt cells; Fig. S1). Results are representative of three controls, seven patients with treated CD, and six patients with active CD. (C) IgA overexpression observed at the apical pole of epithelial cells of patients with active CD was located inside the cell, including the brush border membrane, as shown by its colocalization with cytokeratin and alkaline phosphatase, a marker of apical membranes. No such colocalization was seen in controls. Bars, $50 \mu\text{m}$.

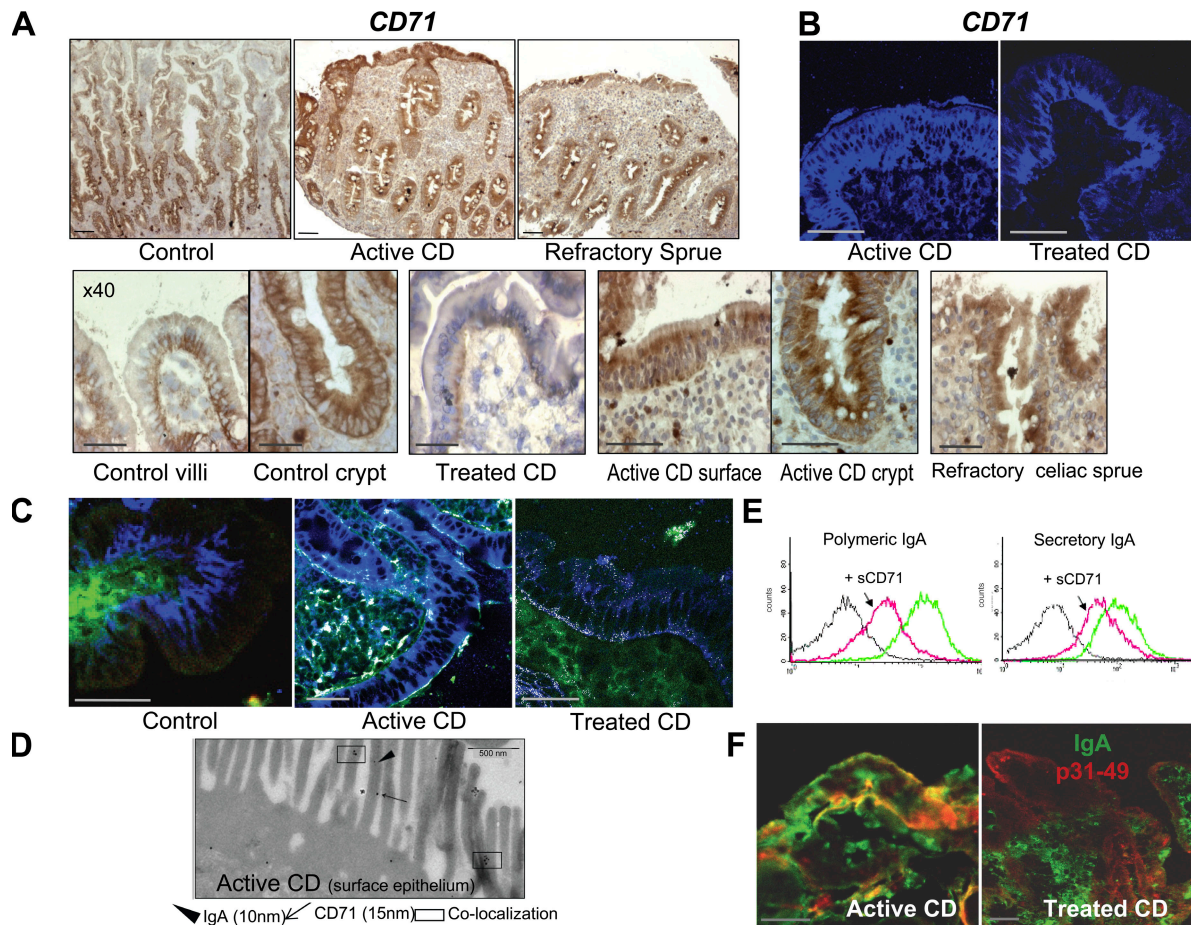


Figure 2. CD71 expression and colocalization with IgA and p31-49 in duodenal biopsies. (A) Expression of CD71 (immunoperoxidase labeling) on duodenal biopsies from controls, patients with active or treated CD, and patients with refractory celiac sprue. Compared with controls, CD71 was overexpressed in patients with active CD and in patients with refractory celiac sprue. At higher magnification (bottom), strong CD71 expression was observed all over the surface epithelium in patients with active CD, whereas in controls and treated CD patients CD71 expression was only observed at the basal pole of villous epithelial cells and in crypt cells. (B) CD71 overexpression by surface epithelium of patients with active CD was confirmed by immunofluorescent labeling. The fluorophore was a cy5-conjugated secondary antibody (blue staining). Results in A and B are representative of three control subjects, four treated CD patients, eight patients with active CD, and two patients with refractory celiac sprue. (C) Double immunofluorescence labeling of IgA-CD71 in duodenal biopsies. Colocalization (white) of IgA (green) and CD71 (blue) was observed at the apical surface of the epithelium in active CD ($n = 5$), but not in controls or in treated CD patients ($n = 3$). (D) Immunogold electron microscopy with double labeling of IgA (10-nm particles; arrowhead) and CD71 (15-nm particles; arrow). In active CD, IgA and CD71 were expressed in the brush border membrane and subepithelial compartments, and IgA-CD71 co-localization was frequent (boxes). No such colocalization was observed in controls (see additional images of one control subject and two patients with active CD in Fig. S2). (E) SIgA can bind CD71 at the cell surface of a B cell line (Daudi cells) known to express CD71 as the only IgA receptor. Cells were incubated for 30 min at 4°C with 500 $\mu\text{g}/\text{ml}$ SIgA or pIgA1 in the presence or absence of 500 $\mu\text{g}/\text{ml}$ of soluble CD71. IgA was revealed with biotinylated anti-IgA and allophycocyanin-labeled streptavidin (green line). Both pIgA1 and SIgA specifically bound CD71, as the binding was inhibited by soluble CD71 receptors (pink line). The black line indicates the isotope control. (F) Colocalization (yellow) of IgA (green) and p31-49 (red) in duodenal biopsies from two patients with active CD, mounted in Ussing chambers and exposed to p31-49-TAMRA on the apical side for 15 min at 37°C before being fixed, cryosectioned, and stained with anti-IgA-FITC antibodies. No colocalization was found in two treated CD patients or in a control (not depicted). Bars, 50 μm .

binding was inhibited by prior incubation of IgA with soluble CD71 receptors (Fig. 2 E), acting as a competitive inhibitor.

Finally, colocalization of IgA (green) and p31-49-tetramethyl-6-carboxyrhodamine (TAMRA; red) was studied after applying the peptide for 15 min at 37°C on the apical side of biopsies mounted in Ussing chambers. As shown in Fig. 2 F, colocalization of p31-49 and IgA was detected in biopsies from patients with active CD but not in those from treated CD patients (or controls; not depicted). The lack of IgA colocal-

ization with p31-49 in treated patients was compatible with total degradation of the peptide during intestinal transport (Fig. 3 A). The red fluorescence observed in the lamina propria of treated CD patients (and, to a lesser extent, in patients with active CD) is likely related to the release of the TAMRA fluorescent label after endocytosis and hydrolysis of p31-49 by epithelial cells. Collectively, these results support the hypothesis that CD71 may bind SIgA-gliadin peptide complexes at the apical surface in active CD.

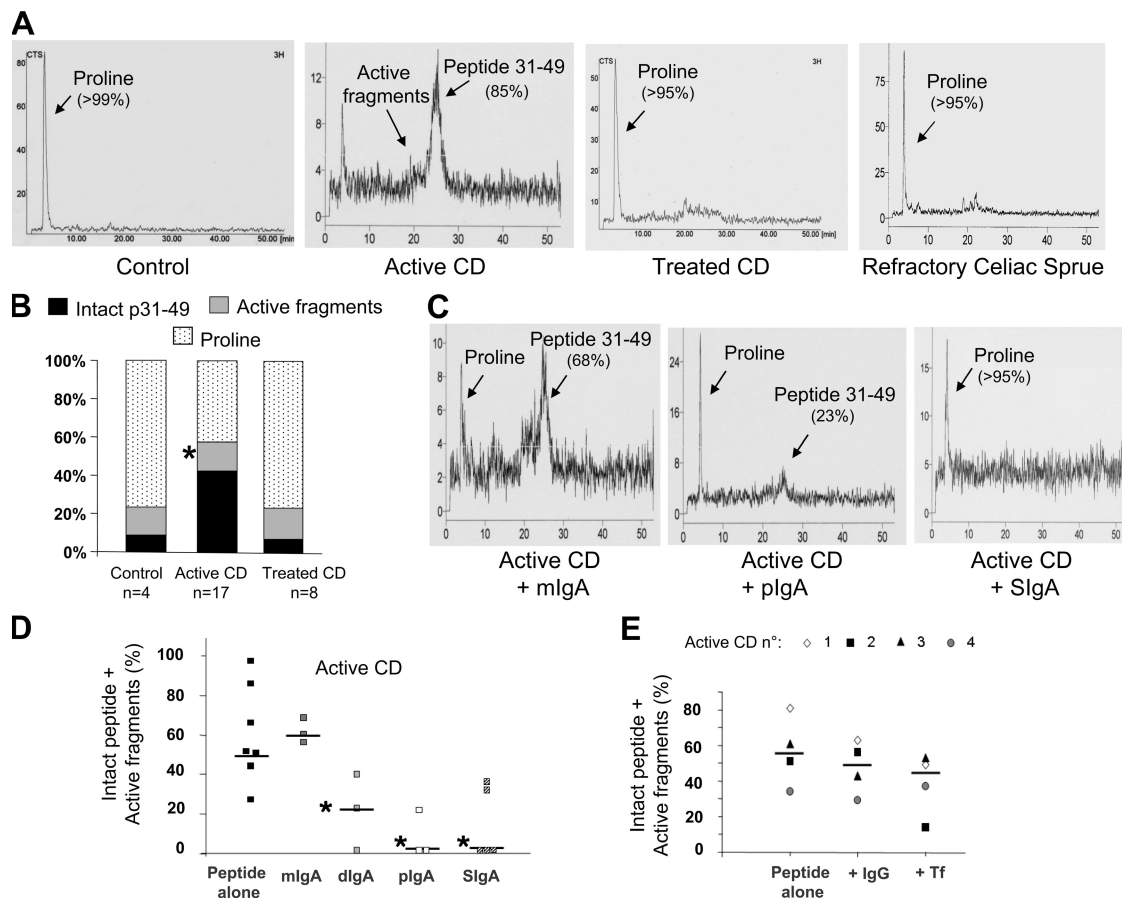


Figure 3. IgA involvement in intestinal transport and processing of ^3H -labeled p31-49. (A) Transport and processing of p31-49 showing typical RP-HPLC elution pattern of ^3H -labeled material in the basal compartment of duodenal biopsies incubated for 3 h after apical addition of ^3H -labeled p31-49. In controls and treated CD patients, p31-49 was almost completely degraded during transport, as >95% of the total radioactivity was eluted as free ^3H -labeled proline in the basal compartment. In contrast, in patients with active CD ($n = 7$), a large fraction of p31-49 was found on the basal side of the intestinal mucosa, mainly in intact form or as active fragments. Interestingly, in three patients with refractory celiac sprue (flat mucosa and an absence of antigliadin IgA), near-complete degradation of the peptide was observed after intestinal transport, suggesting that a flat mucosa is not responsible for the transport of intact peptide observed in patients with active CD. (B) Mean percentage of tritiated intact p31-49, active fragments, and proline found in the basal compartment after intestinal transport of p31-49 by duodenal biopsies from controls, patients with treated CD, and patients with active CD. The percentage of intact p31-49 plus active fragments crossing the duodenal biopsies (mean \pm SD) was significantly higher in active CD ($57 \pm 18\%$; $n = 17$) than in treated CD ($23 \pm 23\%$; $n = 8$) and controls ($26 \pm 4\%$; $n = 4$). *, $P < 0.007$. (C) Inhibitory effect of pIgA, mIgA, and sIgA on the transport of intact p31-49. To test the involvement of IgA in the transport of intact p31-49, we performed competitive inhibition experiments with different forms of IgA. In a typical RP-HPLC elution profile of ^3H -labeled p31-49 obtained in biopsies from the patient with active CD shown in A, 85% of p31-49 was transported intact in basal conditions, whereas this percentage fell sharply in the presence of pIgA and sIgA but not mIgA (mIgA does not bind significantly to CD71; reference 31). (D) Intestinal transport of p31-49 in patients with active CD showing the percentage of intact p31-49 plus active fragments found in the basal compartment of Ussing chambers after blockade with mIgA, dIgA, pIgA, or sIgA. Compared with "peptide alone" (median = 50; $n = 7$), dIgA (median = 23; $n = 3$), pIgA (median = 0; $n = 3$), and sIgA (median = 0; $n = 5$), but not mIgA (median = 60; $n = 3$), significantly inhibited the intestinal transport of p31-49. *, $P < 0.01$ compared with peptide alone. (E) Effect of IgG and Tf on the transport of intact p31-49. 50 $\mu\text{g}/\text{ml}$ IgG and 10 $\mu\text{g}/\text{ml}$ Tf were preincubated for 30 min on the apical side of duodenal biopsies mounted in Ussing chambers before adding ^3H -labeled p31-49. The basal compartment was collected after 3 h and analyzed by radio RP-HPLC to detect p31-49 and its metabolites. No inhibitory effect on p31-49 transport was observed.

IgA mediates intestinal transport of intact p31-49 through CD71

To directly address the role of IgA in the protected apical to basal transport of gliadin peptides, duodenal biopsies were mounted in Ussing chambers and ^3H -labeled p31-49 was applied to the apical side. Intestinal transport was evaluated by analyzing ^3H -labeled fragments in the basal compartment 3 h later. Confirming our published results (15), a large fraction of

^3H -labeled p31-49 was transported intact across the duodenal mucosa of patients with active CD who have both a flat mucosa and IgA lining the surface epithelium (Fig. 3 A). In contrast, this peptide was almost totally degraded during transport through samples from control subjects and treated CD patients who had a normal intestinal epithelium with much less CD71 and IgA than patients with active CD. Furthermore, the peptide was similarly degraded during transport across duodenal biopsies

from three patients with refractory celiac sprue, a complicated form of CD associated with severe villous atrophy, CD71 apical overexpression but no detectable IgA on the epithelial cell surface (Fig. 1 B and Fig. 3 A). The latter result indicates that nonspecific leakage caused by epithelial flattening is not sufficient to explain the increased transport of the intact peptide observed in active CD. The percentage of intact p31-49 and active fragments found in the basal compartment in active CD is significantly higher than that observed in controls and treated CD patients (Fig. 3 B). Adding an excess of dIgA, pIgA, or SIgA to the apical compartment of duodenal biopsies from patients with active CD, as competitive inhibitors of endogenous gliadin-specific IgA, strongly inhibited the transport of intact p31-49 (Fig. 3, C and D), indicating that large IgA molecules are involved in this transport. In contrast, monomeric IgA (mIgA) had no inhibitory effect, as expected in view of its low affinity for CD71 (31).

To better understand the mechanism underlying intestinal transport of p31-49, we tested potentially inhibitory molecules.

IgG and human Tf were unable to inhibit the protected transport pathway (Fig. 3 E), indicating that gliadin-specific IgG does not play a major role in the intestinal absorption of gliadin peptides in patients with active CD. The failure of Tf to inhibit p31-49 transport is in line with its inability to inhibit IgA binding to CD71 on Daudi cells (31) and suggests that IgA and Tf do not share the same binding site on CD71. In addition, the anti-CD71 mAb A24 did not inhibit the transport of intact p31-49 in active CD, suggesting that A24, like Tf, does not bind CD71 at the same site as IgA (Fig. S3 A, available at <http://www.jem.org/cgi/content/full/jem.20071204/DC1>) (32). Also, an mAb directed to SC was not able to block the transport of IgA-p31-49 complexes (Fig. S3 B). Finally, we used a mAb to Tgase II (anti-Tgase II, 6B9) or dansyl cadaverin, an inhibitor of Tgase activity, to test the involvement of Tgase II, and no inhibitory effect was observed.

To confirm the role of CD71 in the transport of intact p31-49, we tested the capacity of a soluble CD71 receptor to competitively inhibit this transport in duodenal biopsies from six

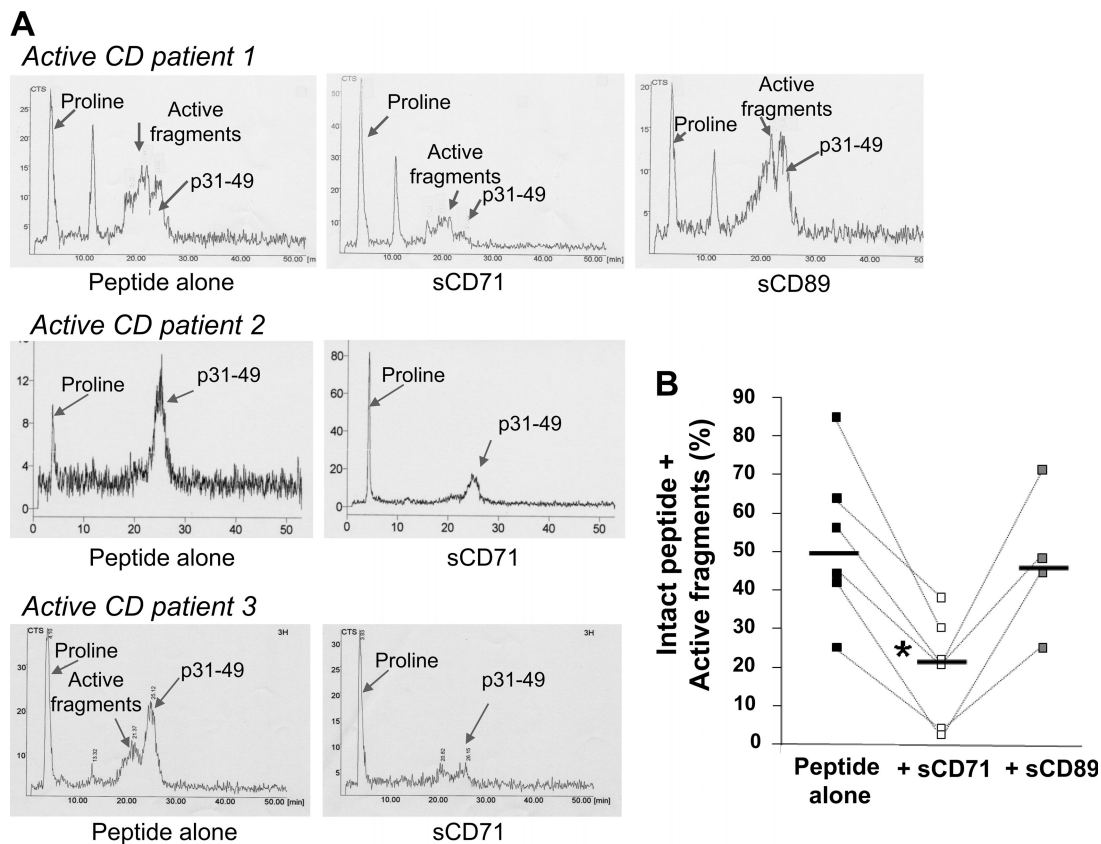


Figure 4. Inhibitory effect of soluble CD71 on the transport of intact p31-49. (A) Typical HPLC elution profiles of p31-49 after intestinal transport across duodenal biopsies from patients with active CD. ^3H -labeled radioactive material is present in the basal compartment of the duodenal biopsies in Ussing chambers 3 h after adding ^3H -labeled p31-49 to the apical compartment. In basal conditions, intact p31-49 or active fragments were present in the basal compartment. Soluble CD71 (sCD71) reduced the transport of intact p31-49, whereas soluble CD89 (sCD89) had no effect. (B) Inhibition of intestinal transport of ^3H -labeled p31-49 in the presence of sCD71. The histogram shows the mean percentage of intact p31-49 and its active fragments found in the basal compartment after intestinal transport. Compared with peptide alone (median = 50; $n = 6$), significant inhibition was observed in the presence of 30 $\mu\text{g}/\text{ml}$ sCD71 (median = 21; $n = 6$) but not sCD89 (median = 46; $n = 4$). The horizontal lines indicate median values, and dotted lines join paired results from the same patient. *, $P < 0.01$ compared with peptide alone.

patients with active CD using Ussing chambers. When soluble CD71 receptor was added to the apical compartment, reversed-phase HPLC (RP-HPLC) analysis of the radioactive material recovered in the basal compartment after intestinal transport of ^3H -labeled p31–49 showed that transport of the intact peptide was significantly inhibited (Fig. 4, A and B). In contrast, soluble CD89 receptor had no inhibitory effect, in accordance with the absence of CD89 expression in epithelial cells.

IgA mediates the intestinal transport of intact 33-mer via CD71

Intestinal transport of 33-mer in the form of the intact peptide and large immunogenic 12-mer (16) fragments (35 and 28%, respectively) was significantly higher in active CD than in controls (4 and 9%) and in treated CD (12 and 8%) (Fig. 5, A and B, left), confirming our previous results (15, 16). In active CD, competitive inhibition of 33-mer transport was attempted with

dIgA, pIgA, and soluble CD71 (Fig. 5 B, right). The percentage of 33-mer crossing the intestinal mucosa in intact form was significantly reduced by dIgA and pIgA (7 and 14%, respectively) and by soluble CD71 (19%) compared with the peptide alone (46%; $P < 0.01$), further supporting CD71 mediation of protected IgA–33-mer complex retrotransport.

DISCUSSION

This study reveals that protected transport of gliadin peptides in CD is driven by retrotranscytosis of SIgA through the Tf receptor (CD71), which is abnormally expressed on the apical side of the intestinal epithelium. This process may sustain anti-gliadin immune responses and aggravate intestinal inflammation in CD patients.

Ig-mediated transport of luminal antigens across the epithelium has already been reported in various situations. Protected apical to basal intestinal transport of IgE–allergen complexes

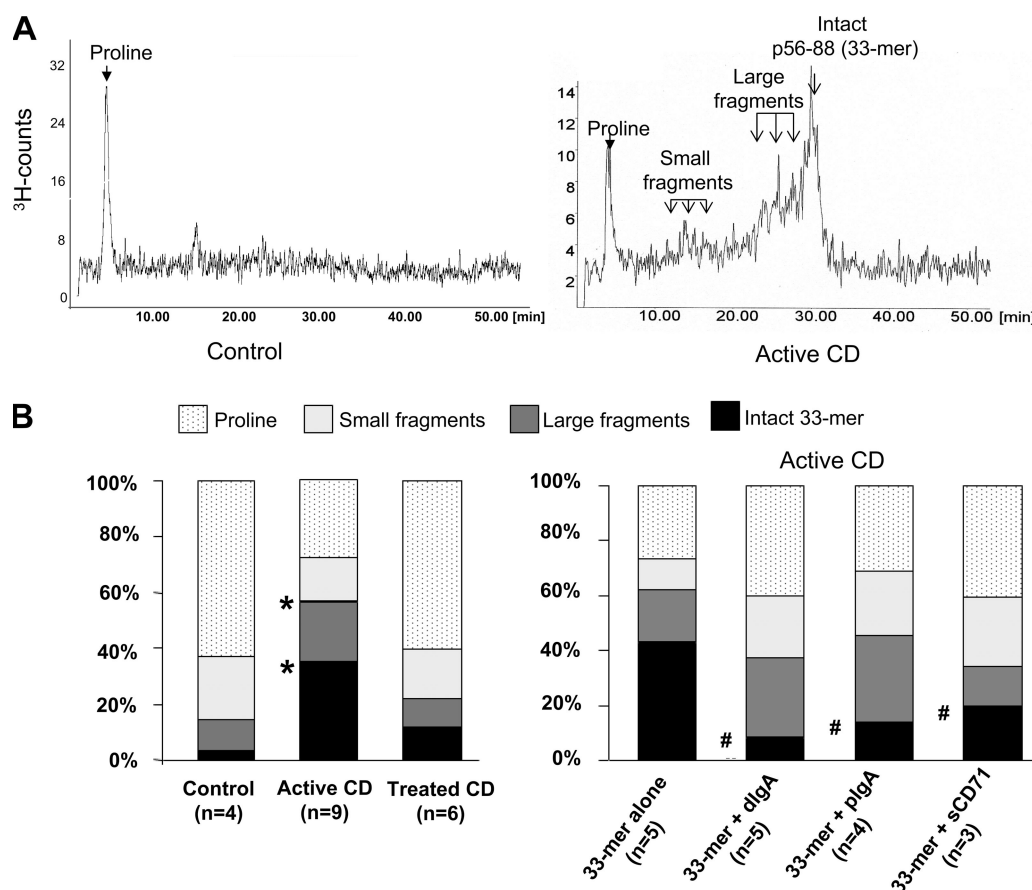


Figure 5. Duodenal transport of 33-mer in patients with active CD, and competitive inhibition by IgA and soluble CD71. (A) Typical RP-HPLC elution profile of ^3H -labeled 33-mer after intestinal transport across duodenal biopsies from a control individual and a patient with active CD, mounted in Ussing chambers. ^3H -labeled radioactive material present in the basal compartment 3 h after adding ^3H -labeled 33-mer to the apical compartment is shown. The percentages of the different eluted fractions (proline, small and large fragments, and intact 33-mer) were quantified with Radiostar software. The control tissue almost totally degraded the 33-mer peptide, whereas digestion was incomplete in the sample from the patient with active CD. (B, left) Mean percentages of 33-mer and its fragments after intestinal transport. The duodenal mucosa of patients with active CD does not completely degrade 33-mer, as 38 and 22% of intact peptide and large fragments, respectively, were recovered in the basal compartment, compared with 4 and 9% in control subjects. Treated CD patients had an intermediate profile (12 and 8%). (right) dIgA, pIgA, and soluble CD71 (sCD71) significantly inhibited the transport of intact 33-mer but not of large fragments. *, $P < 0.04$ compared with control; #, $P < 0.04$ compared with peptide alone.

through CD23, the low-affinity IgE receptor, has been shown in allergic patients and might elicit rapid activation of intestinal mast cells (33–35). Comparable protected transport of IgG–antigen complexes via the neonatal Fc receptor has also been demonstrated. This receptor, initially described at the apical surface of enterocytes in newborn rodents (36) and human fetal intestine (37, 38), could transport IgG into the intestinal lumen and recycle IgG–antigen complexes back into the lamina propria, thereby promoting a specific immune response (38).

In this study, we tested the hypothesis that, in active CD, SIgA–gliadin immune complex retrotransport could be mediated by CD71 and, thus, explain the protected transport of gliadin peptides in celiac patients. In healthy individuals, vectorial intestinal transport of IgA consists mainly of basal to apical transcytosis of dIgA via the pIgR, leading to the release of SIgA in the intestinal lumen (39), where it retains microbial and food antigens and confers protective mucosal immunity. Although basal to apical transfer of IgA is the norm in epithelia, apical to basal retrotransport of SIgA through M cells overlying Peyer's patches has been documented in mice (40, 41) even though specific IgA receptors are not identified. Several lines of evidence support retrotransport of SIgA–gliadin complexes in active CD. First, the intestinal lumen of patients with active CD contains elevated levels of antigliadin IgA antibodies (17), which can bind a set of gliadin peptides (42, 43) including the 33-mer (44) and p31–55 (45). We show the presence of high molecular weight gliadin-specific IgA immune complexes containing Tgase. This is an important finding, as IgA in form of high molecular weight complexes binds CD71 with high affinity (31). Second, we show that IgA is more strongly concentrated at the apical pole of surface epithelial cells in active CD than in treated CD patients, refractory celiac patients, and control subjects, and that this pattern correlates with substantial transport of intact p31–49 and 33-mer in active CD, whereas this transport is negligible in controls, treated CD patients, and patients with refractory celiac sprue. Finally, IgA involvement in the protected transport of these gliadin peptides is indicated by the competitive inhibition of their transport by pIgA observed in this study. Although inhibition of 33-mer transport by IgA concerns the intact peptide only and not its large 12-mer fragments, these latter fragments have been shown to stimulate T cells much less potently than the intact peptide (6). One possible explanation is partial resistance of 33-mer to lysosomal degradation during fluid-phase transcytosis occurring in parallel with receptor-mediated transcytosis.

A role of IgA in protected transcytosis of gliadin peptides would imply the presence of a receptor able to bind pIgA/SIgA at the apical surface of enterocytes. Our findings highlight the role of the Tf receptor CD71, a newly identified IgA receptor. Previous studies have shown that CD71 binds pIgA but not mIgA (31). In this study, we show that CD71 can also bind SIgA, the main form of IgA present in the intestinal lumen. In the normal intestine, CD71 is mainly expressed in crypts (restricted to the basolateral membrane of epithelial cells) (46) with little expression in the villous epithelium. In active CD, villous flattening, increased epithelial renewal, and iron-deficiency

anemia are all associated with substantial CD71 up-regulation all over the surface epithelium, and this may be responsible for its missorting toward the apical pole of enterocytes. This is compatible with a role of CD71 in the endocytosis of IgA complexes from the apical cell surface. Previous studies have shown in nonpolarized cells that pIgA binding to CD71 induces the internalization and rapid addressing of IgA-loaded CD71 into recycling vesicles (31). The colocalization that we observed between IgA and CD71 and between IgA and p31–49 in active CD but not in controls suggests that IgA–gliadin complexes might bind CD71. These findings, combined with the demonstration that soluble CD71 receptors (as well as dIgA, pIgA, and SIgA) can block the protected transcellular transport of intact p31–49 and 33-mer, provide strong evidence that CD71 is the receptor that allows gliadin peptides bound to SIgA to translocate from the intestinal lumen into the lamina propria in active CD. Interestingly, the capacity of CD71 to mediate pIgA binding to mesangial cells of patients with IgA nephropathy also underlines the pathogenic role of CD71–IgA interactions in IgA nephropathy (19, 31). The presence of glomerular IgA deposits in a significant proportion of newly diagnosed CD patients (47) and the abnormally elevated incidence of CD in patients with IgA nephropathy (48) provides a link between these diseases.

The specific retrotranscytosis of SIgA–gliadin complexes in CD may appear puzzling given the presence of SIgA with diverse specificities in the intestinal lumen. First, large IgA complexes bind CD71 with higher affinity than smaller IgA species such as SIgA (31). Second, SIgA–gliadin complexes could be selectively retrotranscytosed with the help of tissue Tgase, which we detected in the high molecular weight IgA immune complexes found in duodenal secretions. Indeed, Tgase can cross-link gliadin peptides and promote receptor-mediated endocytosis (49), particularly the internalization step of CD71 (50), and was recently detected at the surface of enterocytes in active CD (51). However, neither Tgase antibodies nor an inhibitor of Tgase activity could inhibit the protected transport pathway in biopsy specimens from patients with active CD, suggesting that Tgase is not directly involved in the transport process or that Tgase effect is irreversible in biopsies from patients with active CD mounted in Ussing chambers. Finally, the pIgA and SIgA concentrations used in our Ussing chamber experiments were high enough, in view of the small exposed surface area (0.025 cm²), for competitive inhibition of IgA–gliadin transport to occur.

Gliadin-specific SIgA is present at high titers in the intestinal lumen of patients with active CD but is also found in healthy individuals (52, 53). In contrast, apical to basal delivery of intact gliadin peptides is only observed in active CD. Our data indicate that CD71 overexpression and misaddressing to the apical pole of enterocytes is a key event in the intestinal retrotransport of SIgA–gliadin complexes (Fig. 6). They also indicate that the normal function of SIgA, namely the containment of harmful antigens in the intestinal lumen, is deficient in CD. This could account for the abnormal immune response to gluten in genetically susceptible (HLA-DQ2/8) individuals.

It is unclear whether this abnormal transport is the triggering event in CD or whether it becomes operational secondarily,

perpetuating inflammation once the mucosa has flattened. Several environmental factors may serve as initial triggers for SIgA–gliadin complex entry into intestinal tissue. Among these factors, iron deficiency anemia, by inducing CD71 up-regulation, could promote the delivery of the SIgA–gliadin complex, triggering abnormal intestinal responses in susceptible individuals. In addition, strong intestinal epithelial cell proliferation secondary to epithelial damage by intestinal infection might also stimulate CD71 overexpression and thereby favor the onset of CD in susceptible individuals. It is noteworthy that frequent rotavirus infection is associated with a higher risk of autoimmunity, defined as positivity for tissue Tgase, in early childhood (54).

Finally, if CD71 is necessary for CD to develop, why do patients with treated CD react so rapidly to the ingestion of gluten in the absence of CD71 expression? Some CD patients on a gluten-free diet still have minor small bowel abnormalities, and there is evidence that the celiac epithelium may be persistently activated even after successful treatment and mucosal healing (55). It is possible that residual expression of CD71 at the apical membrane of enterocytes may drive the entry of small amounts of gliadin peptides that are nonetheless sufficient to reactivate gluten-sensitive memory T cells and participate in rapid relapse.

In conclusion, we describe a novel mechanism of CD71-mediated IgA transcytosis that enables gliadin peptides to enter the lamina propria of patients with active CD. Inhibition of

this protected transport pathway might provide a new therapeutic option, blocking the cascade that perpetuates innate and adaptive immune responses to gluten in patients with active CD.

MATERIALS AND METHODS

Patients

We studied 26 patients with active CD, 13 treated CD patients who had been on a gluten-free diet for at least 1 yr, 4 patients with refractory celiac sprue, and 10 nonceliac control subjects. Diagnosis of active CD was based on subtotal or total villous atrophy and positivity for antigliadin, anti-Tgase, and antiendomysium IgA antibodies. Refractory celiac sprue was defined as CD resistant to a gluten-free diet, characterized by the persistence of villous atrophy in the absence of antigliadin IgA antibodies. The control subjects underwent upper endoscopy for routine diagnostic purposes (e.g., dyspepsia and chronic diarrhea) and had a normal intestinal mucosa. All the patients underwent duodenal endoscopy, during which four to six additional biopsy samples were taken from the distal duodenum for research purposes. In some cases, duodenal secretions were obtained by infusion–aspiration of 50 ml of phosphate-buffered saline. Local ethics committee (Comité de Protection des Personnes Ile de France II) approval was obtained, and all of the patients signed an informed consent to participate in the study.

Purification of IgA and synthesis of soluble IgA receptors

Competing IgA used in the Ussing chamber studies was human myeloma IgA1 protein prepared according to Chevailler et al. (56). mIgA, dIgA, and pIgA were separated by gel filtration on columns (>98% pure; Sephacryl S300 [GE Healthcare]) (57). SIgA consisting of pooled human colostrum was obtained from Fitzgerald or Biotrend. The soluble IgA receptors sCD71 (TfR) and sCD89 were used. Both soluble receptors were expressed in a lytic baculovirus/insect cell expression system, as described previously (19).

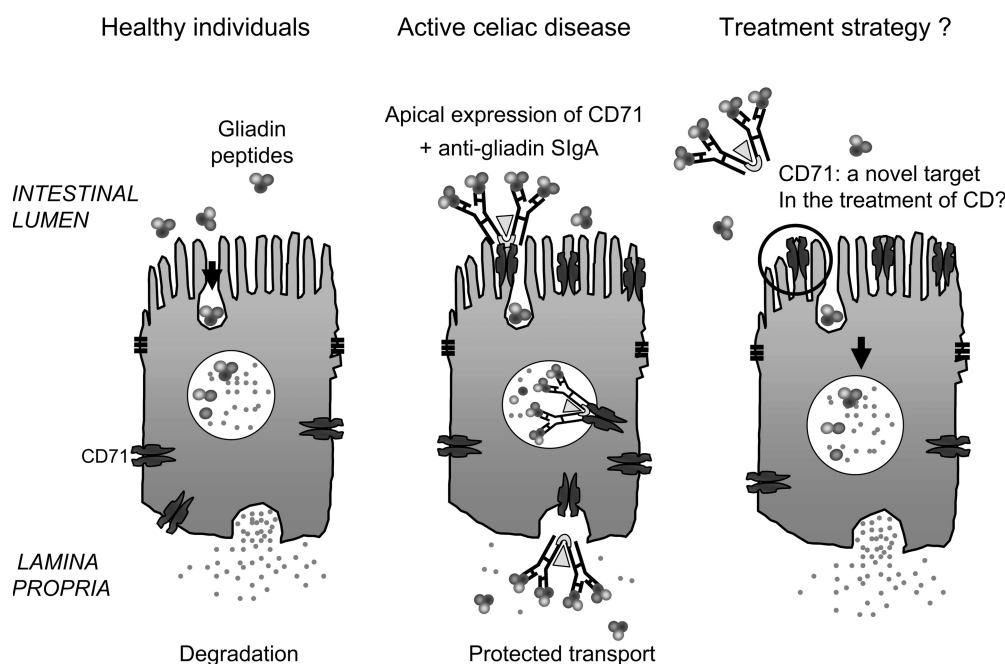


Figure 6. Overview of postulated Tf receptor (CD71)-mediated transport of IgA–gliadin complexes in CD. In healthy individuals, gliadin peptides (resistant to luminal degradation) are taken up nonspecifically by enterocytes and are degraded by lysosomal acid proteases during fluid-phase transcytosis. Very few toxic peptides are delivered into the intestinal lamina propria. In patients with active CD, abnormal expression of CD71 (Tf receptor) at the apical pole of enterocytes allows receptor-mediated uptake of SIgA–gliadin peptide complexes and their protected transport toward the lamina propria and, thus, toward the local immune system. The exact part of the SIgA molecule involved in CD71 binding is not known. Blockade of gliadin peptide entry into the intestinal mucosa might serve as the basis for a novel therapeutic strategy in CD.

radioactivity eluted in each peak were performed with Radiostar software (Berthold). In this setting, free ^3H -labeled proline (the radiolabeled amino acid in the peptide sequence) is eluted with a retention time (Rt) of 4 min, whereas intact p31-49 and 33-mer are eluted at 24.5 and 30 min, respectively. As already reported (15), there was no degradation of p31-49 or 33-mer in the apical compartment after 3 h of incubation. Radio-RP-HPLC analysis of the basal compartments allowed us to evaluate p31-49 and 33-mer degradation during their intestinal transport by calculating the relative percentage of intact peptides and their degradation fragments. Our previous mass spectrometry studies (16) showed that p31-49 fragments eluting between 17 and 21 min (named "active fragments") correspond mainly to p31-43, a toxic sequence of p31-49 (11), and that large 33-mer fragments eluting between 23 and 28 min correspond to immunostimulant 12-mer peptides (named "large fragments").

Statistical analyses

The results are reported as means \pm SD or as scatter plots and medians. Multiple comparisons (analysis of variance) followed by group-to-group comparisons were performed with the general linear model procedure or the nonparametric Wilcoxon test of the SAS software package (SAS Institute). Differences were considered to be statistically significant at $P < 0.05$.

Online supplemental material

Fig. S1 examines IgA expression in crypt and surface epithelium of control subjects and active celiac patients. Fig. S2 presents an immunogold electron microscopy study of IgA and CD71 expression or colocalization in duodenal biopsies from one control subject and two patients with active CD. Fig. S3 describes the attempted inhibition of intact ^3H -labeled p31-49 transport in active celiac patients in the presence of anti-CD71 mAb, anti-SC mAb, anti-Tgase II mAb, or the Tgase inhibitor dansyl cadaverin. Online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20071204/DC1>.

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