CD98-mediated Links between Amino Acid Transport and β₁ Integrin Distribution in Polarized Columnar Epithelia*

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In non-polarized cells, CD98 has been shown to both influence β₁ integrins and heterodimerize with LAT-2, which confers amino acid transport capability on the LAT-2/CD98 heterodimer. Since LAT-2 is most heavily expressed in intestine and CD98 associates with the β₁ integrin splice form selectively found in such epithelia, we investigated the relationship and polarity of these proteins using the intestinal epithelial model Caco2-BBE. CD98 was found to selectively communoprecipitate with both LAT-2 and β₁ integrin, and, logically, all three proteins were polarized to the same (basolateral) domain. Furthermore, expression of CD98 in polarized epithelia lacking human CD98 (MDCK cells) disrupted β₁ integrin surface distribution and cytoskeletal architecture, suggesting that CD98 can influence integrin function. Expression of a CD98 mutant lacking the specific residues conferring LAT-2 binding similarly affected cells, confirming that the latter effect was not due to LAT-2 sequestration. Use of CD98 truncation mutants suggest that a 10-amino acid domain located at the putative cytoplasmic tail/transmembrane domain interface was necessary and sufficient to induce the phenotype change. We conclude that the CD98/LAT-2 amino acid transporter is polarized to the same domain on which β₁ integrin resides. CD98 appears to associate with β₁ integrin and, in doing so, may influence its function as revealed by disruption of the outside-in signaling that confers cytoskeletal organization. Furthermore, such findings suggest a link between classic transport events and a critical element of barrier function: integrin-mediated influences on cytoskeletal organization.

Polarized epithelial cells contain distinct apical and basolateral membranes with unique protein and lipid composition. The asymmetric distribution of plasma membrane components is a fundamental characteristic of epithelial cells (1). For example, the ability of an epithelium to secrete or absorb fluid is closely linked to the asymmetric distribution of ion transport processes within its membranes (2–4). Recently, a growing body of both experimental and clinical evidence indicates that surface adhesion molecules, such as integrins, are required for normal epithelial development and that the mutation or absence of these molecular adhesins may lead to deranged growth control and/or altered epithelial cell function and cell polarity (5). Because epithelial cells rest on the extracellular matrix (ECM), it is logical to expect specific interactions between basolateral “receptors” such as integrins and ECM. Indeed, upon binding to ECM ligands (outside), integrins deliver signals that control cell proliferation, gene induction, differentiation, and polarization (6).

It has been suggested that integrin function is readily modulated by various proteins and protein complexes, including oncogenes (7). Recently, it has been shown that CD98, a cell surface protein formed by covalent linkage of CD98 heavy chain (CD98hc) with several different light chains to form amino acid transporters, also functions as a chain (CD98hc) with several different light chains to form amino acid transporters, also functions as a

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1 The abbreviations used are: ECM, extracellular matrix; PBS, phosphate-buffered saline; MDCK, Madin-Darby canine kidney; FACS, fluorescence-activated cell sorting; PCR, polymerase chain reaction; kb, kilobase pair(s); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PAGE, polyacrylamide gel electrophoresis; HBSS, Hank’s balanced salt solution.
that the heterodimer CD98/LAT-2 (the functional amino acid transporter) is polarized to the basolateral domain of Caco2-BBE monolayers. Moreover, CD98 communoprecipitates with \( \beta_1 \) integrin as well as with LAT-2. Furthermore, overexpression of the heterodimeric or monomeric human CD98 cDNA in a polarized epithelial cell line prevents normal \( \beta_1 \) integrin surface distribution and thus prevents the outside-in signaling that permits actin cytoskeletal organization. Since this occurs even in cells lacking the appropriate LAT-2 binding domain for CD98, it is likely that this reveals a LAT-2-independent CD98-mediated regulation of epithelial \( \beta_1 \) function. Using various CD98 truncation constructs, we identify a 10-amino acid domain located in the cytoplasmic tail/transmembrane portion of the human CD98 that is necessary and sufficient to induce the observed phenotypic change. Such findings suggest a linkage between the epithelial functions of transport and polarity/barrier distribution and thus prevents the outside-in signaling that permits actin cytoskeletal organization.

### MATERIALS AND METHODS

**Cell Culture**—Caco2-BBE (17) or MDCK (ATCC) were grown as confluent monolayers in a 1:1 mixture of Dulbecco’s Vogt modified Eagle’s medium and Ham’s F-12 medium supplemented with 15 mM HEPES buffer, pH 7.5, 14 mM NaHCO\(_3\), and 10% newborn calf serum. Monolayers were subcultured every 7 days by trypsinization with 0.1% trypsin and 0.02 mM EDTA in Ca\(^{2+}\)/Mg\(^{2+}\)-free phosphate-buffered saline (PBS). Transfected cell lines were maintained in the same media containing 1.2 mg/ml G418 (Sigma). Clones viewed under light microscope were selected and trypsinized with cloning rings. The 10 clones selected from each construct were maintained in 1.2 mg/ml G418 and were expanded. Clones showing the highest wild type, mutated, and deleted human CD98 expression by FACS and Northern analysis were selected for this study.

**Generation of Polyclonal Antibodies to Human LAT-2**—Based on a computerized predictive model for antigenicity and uniqueness (favorable secondary structure, hydrophathy, peptide location, and lack of homology with other proteins in the data base), a synthetic peptide was designed, EVERGSGTEEANEDME, corresponding to residues 497–512 of the deduced LAT-2 protein. The peptide was coupled to keyhole limpet hemocyanin, and anti-LAT-2 antibody was raised in rabbits following a standard 80-day immunization protocol. The reactivity of the resulting antiserum against the peptide was tested by enzyme-linked immunosorbent assay, and the antibody was affinity-purified against the synthetic peptide. Antibody yields were as follows: rabbit 1, 2 mg/ml \( \times 7.6 \) ml; rabbit 2, 2 mg/ml \( \times 12.8 \) ml. Antibody from rabbit 2 was used for the present study.

**Northern Blot Analysis**—Total RNA (20 \( \mu \)g) from Caco2-BBE or MDCK cells was denatured, subjected to electrophoresis on a 1.2% agarose gel containing formaldehyde, transferred to a nylon membrane (PerkinElmer Life Sciences), and hybridized with an \(^{32}\)P-labeled 5'-triphasate-labeled, randomly primed 1.7-kilobase (kb) (full-length) CD98 cDNA probe, 0.47-kb (1261–1730) LAT-2 cDNA probe generated by reverse transcription-PCR from Caco2-BBE total RNA, or 0.30-kb (3323 to 3814) \( \beta_1 \) integrin. A GAPDH cDNA probe was used as control (Ambion).
Flow Cytometry—Adherent monolayers were detached with 1 mM EDTA/EGTA in HBSS (without Ca\(^{2+}\) and Mg\(^{2+}\)), pelleted by centrifugation, and resuspended in HBSS containing 0.5% bovine serum albumin. For cell surface CD98, LAT-2, and β\(_1\) integrin molecule expression, Caco2-BBE or MDCK cells were detached with EDTA/EGTA in HBSS (without Ca\(^{2+}\) and Mg\(^{2+}\)). Approximately 5 \times 10^6 cells were treated with saturating amounts (10 μg/ml) of mouse monoclonal antibody specific for CD98 molecules (UM7F8, Ancell), rabbit polyclonal antibody against LAT-2, and mouse monoclonal antibody for β\(_1\) integrin (Life Technologies, Inc.) in 100 μl of 0.5% bovine serum albumin, PBS for 1 h at 4°C, washed twice, and then the samples were stained with saturating amounts (1 μl/ml) of fluorescein isothiocyanate secondary antibody (F(ab')\(_2\)) fragment of sheep anti-mouse IgG (Sigma) in 100 μl for 1 h at 4°C. After washing twice in PBS, 4,000 intact cells (gated on forward and side light scatter parameters) were assayed in a fluorescence flow cytometer (Becton Dickinson).

Western Blot Analysis—Cells were lysed with a solution of 1% (w/v) Triton X-100 in 20 mM Tris, pH 8.0, 50 mM NaCl, 5 mM EDTA, and 0.2% (w/v) bovine serum albumin supplemented with protease inhibitors. Cells lysates were then boiled in sample buffer (containing 2% SDS and 20% glycerol) without β-mercaptoethanol in non-reducing conditions and with 10 mM β-mercaptoethanol (reducing conditions) at 100°C for 5 min. The samples, which contained 50 μg of cell protein, were separated by SDS-PAGE with 7.5% polyacrylamide gel and transferred overnight at 4°C to 0.2-μm nitrocellulose membranes (Bio-Rad). The blots were blocked 1 h with 5% nonfat dry milk in blocking buffer. After washing with blocking buffer, the blots were incubated for 1 h at room temperature with 1:1000 dilution of a goat anti-CD98 human (RDI). After washing twice for 30 min in nonfat dry milk in blocking buffer, they were further incubated for 1 h at room temperature with anti-goat horseradish peroxidase-conjugated antibody diluted 1:1000. The nitrocellulose was washed twice for 30 min in nonfat dry milk in blocking buffer and then probed using a chemiluminescence system (ECL, Amersham Pharmacia Biotech).

Immunoprecipitation—Cells were washed with ice-cold PBS and then lysed on ice in 1 ml of lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40) containing 1 mg/ml apotinin, 1 mM pepstatin, 2 mM serine proteases. The lysates were centrifuged at 10,000 \(\times g\) for 15 min at 4°C, and the supernatants were subjected to immunoprecipitation and immunoblot analysis. For immunoprecipitation, the supernatants were incubated overnight at 4°C with protein G-agarose suspension (50 μl of beads). The beads were pelleted by centrifugation at 12,000 \(\times g\) for 20 s in a microcentrifuge. Supernatants were transferred to fresh tubes, and the appropriate amount of specific antibody (1:1000 dilution of a goat anti-CD98 (RDI), mouse anti-β\(_1\) integrin (Life Technologies, Inc.), a mouse anti-E-cadherin (RDI), and a rabbit anti-LAT-2) was added and gently rocked for 4 h at 4°C. Subsequently, 50 μl of protein G suspension was added to the mixture and incubated overnight at 4°C. The complexes were collected by centrifugation at 12,000 \(\times g\) for 20 s by microcentrifuge. The beads were washed two times for 20 min with buffer 1 (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40), buffer 2 (50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 1 mM EDTA, 0.1% Nonidet P-40), and buffer 3 (10 mM Tris-HCl, pH 7.5, 0.1% Nonidet P-40). 50 μl of gel loading buffer (1% (w/v) Triton X-100 in 20 ml Tris, pH 8.0, 50 mM NaCl, 5 mM EDTA, and 0.2% (w/v) bovine serum albumin supplemented with protease inhibitors and 2% SDS), was added to the agarose pellet, boiled 5 min at 100°C, subjected to SDS-PAGE, and transferred overnight at 4°C to nitrocellulose membranes. The blots were blocked for 1 h with 5% nonfat dry milk in blocking buffer. After washing with blocking buffer, the blots were incubated for 1 h at room temperature with 1:1000 dilution of a goat anti-CD98 (RDI), mouse anti-β\(_1\) integrin (Life Technologies, Inc.), and a rabbit anti-LAT-2 (amino acid transport).
balanced salt solution with calcium, pH 7.4 (HBSS)

grown on filters and then fixed with 3.7% paraformaldehyde in Hank's

streptavidin-agarose (Pierce) for 24 h at 4 °C.

The protein solution was diluted with 1 ml of lysis buffer and then incubated with streptavidin-agarose (Pierce) for 24 h at 4 °C to bind biotinylated proteins. The protein solution was then boiled in sample buffer containing 2% SDS, 20% glycerol, with or without 10 mM β-mercaptoethanol at 100 °C for 5 min. Proteins were separated by SDS-PAGE and transferred overnight at 4 °C to nitrocellulose membranes. The blots were blocked 1 h with 5% nonfat dry milk in blocking buffer.

After washing with blocking buffer, the blots were incubated for 1 h at room temperature with 1:1000 dilution of a goat anti-CD98, mouse anti-β1 integrin, and a rabbit polyclonal antibody LAT-2. They were further incubated for 1 h at room temperature with anti-rabbit horse radish peroxidase-conjugated antibody diluted 1:1000 and probed using chemiluminescence system (ECL, Amersham Pharmacia Biotech).

Cell Surface Biotinylation—Filter-grown cells were rinsed twice with PBS supplemented with 0.1 mM CaCl2 and 1 mM MgCl2. Basolateral or apical sides of the monolayers were incubated with freshly prepared sulfoxysuccinimidobiotin (Pierce) diluted in the same solution (0.5 mg/ml) for 30 min at room temperature. The reaction was quenched with ice-cold 50 mM NH4Cl, and cells were lysed with a solution of 1% (w/v) Triton X-100 in 20 mM Tris, pH 8.0, 50 mM NaCl, 5 mM EDTA, and 0.2% (w/v) bovine serum albumin supplemented with protease inhibitors.

The protein solution was diluted with 1 ml of lysis buffer and then incubated with streptavidin-agarose (Pierce) for 24 h at 4 °C to bind biotinylated proteins. The protein solution was then boiled in sample buffer containing 2% SDS, 20% glycerol, with or without 10 mM β-mercaptoethanol at 100 °C for 5 min. Proteins were separated by SDS-PAGE and transferred overnight at 4 °C to nitrocellulose membranes. The blots were blocked 1 h with 5% nonfat dry milk in blocking buffer.

Results

Caco2-BBE Cells Express CD98 and LAT-2—The expression of CD98 and LAT-2 mRNA were analyzed by Northern blotting of total RNA from Caco2-BBE cells at high stringency conditions. mRNA of 2.2 kb hybridized with CD98 cDNA (Fig. 1A) and mRNA species of ~5 and 3.7 kb hybridized with LAT-2 cDNA (Fig. 2A) as shown previously (14). CD98 protein expression by these polarized epithelial cells was also identified by both Western blotting and FACS analysis (Fig. 1, B and C). Using the anti-human CD98 antibody, Caco2-BBE cell lysates displayed a single immunoreactive band corresponding to ~90 kDa in the presence of β-mercaptoethanol and ~130 kDa in absence of β-mercaptoethanol (Fig. 1B, lanes 1 and 2). Together these results suggested that CD98 in polarized Caco-2-BBE cells is associated covalently with a ~40-kDa protein via a disulfide bond. LAT-2 mRNA (and protein, Fig. 2B) were also expressed in Caco2-BBE and represented a logical candidate to be the protein associated with CD98.

The CD98/LAT-2 Heterodimer and β1 Integrins Are Basolaterally Polarized—The membrane localization of the human heterodimer CD98/LAT-2 was assessed in confluent Caco2-BBE monolayers. We examined the plasma membrane expression of CD98/LAT-2 by confocal immunofluorescence microscopy and surface biotinylation. As shown by confocal microscopy, β1 integrins, CD98, and LAT-2 had strong lateral membrane and partial basal fluorescence (Fig. 3). In contrast, the apical plasma membrane showed only a slight/questionable fluorescence for β1 integrin, CD98, and LAT-2 (Fig. 3). Further confirmation of our immunofluorescence data was obtained through surface biotinylation of Caco2-BBE monolayers. Plasma membrane domain-specific cell surface membrane glycoproteins were labeled by biotinylation of each plasma membrane domain (apical and basolateral). Western blot using the anti-CD98 displayed one immunoreactive band at ~130 kDa under non-reducing conditions, which are predominantly expressed on the basolateral membrane (Fig. 4B, lanes 1 and 3). Similarly, antibody to β1 integrin reacted with a band at 130 kDa under non-reducing conditions (Fig. 4A, lanes 1 and 2) predominantly detectable on the basolateral membrane in Caco2-BBE monolayers. Under reducing conditions, the anti-CD98 antibody detected an immunoreactive band at ~90 kDa (Fig. 4B, lane 2) exclusively present on the basolateral membrane. We also confirmed the association between CD98/LAT-2 using the anti-human LAT-2 antibody that we generated. Anti-human LAT-2 detected an immunoreactive band at 140 kDa under non-reducing conditions (Fig. 4C, lane 1) and a band at 45 kDa under reducing conditions (Fig. 4C, lane 2), both exclusively present on the basolateral membrane. Together these
results demonstrate that the heterodimer CD98/LAT-2 and β1 integrin are predominantly expressed in the basolateral aspect in Caco2-BBE cell monolayers.

CD98 Associates with Both β1 Integrin and LAT-2—To see if the CD98/LAT-2 heterodimer associates with β1 integrin, immunoprecipitation studies were performed. Caco2-BBE cell lysates were subjected to immunoprecipitation for CD98 (Fig. 5, lane 1), β1 integrin (Fig. 5, lane 2), no antibody (Fig. 5, lane 3), cadherin (Fig. 5, lane 4), or LAT-2 (Fig. 5, lane 5). As shown in Fig. 5, all immunoprecipitates were probed with the CD98 (top panel) or LAT-2 (bottom panel) antibody. CD98 and LAT-2 immunoprecipitates were detected by either CD98 or LAT-2 antibody (Fig. 5, lanes 1 and 5). However, the immunoreactive bands were in a higher molecular complex (−200 kDa) than the expected size of the heterodimer CD98/LAT-2 (−130 kDa), suggesting that the heterodimer CD98/LAT-2 may precipitate in association with additional protein(s).

We tested whether CD98/LAT-2 possibly associates with β1 integrin in Caco2-BBE cells. β1 integrin immunoprecipitates (Fig. 5, lane 2) were probed by either CD98 or LAT-2 antibody; an immunoreactive doublet at 200 kDa was consistently observed (potentially representing splice variants). The immunoreactive bands were in a higher molecular complex (−200 kDa) than the expected size of β1 integrin (−130 kDa), suggesting that the β1 integrin migrates in association with other protein(s). Together these results suggest an association between CD98/LAT-2 and β1 integrin in Caco2-BBE cells.

Overexpression of Human CD98 Alters β1 Integrin Surface Distribution and the Ability to Organize the Actin Cytoskeleton—Our second approach was to examine the effect of overexpression of human CD98 in a polarized cell line. We first selected a polarized cell line that does not express human CD98 but expresses a β1 integrin (closely related to human as it shares similarity in the cytoplasmic domain to which CD98 would necessarily have to interface). As shown in Fig. 6, MDCK cells do not express CD98 mRNA as recognized by the human probe (Fig. 6, lane 1) or a related protein as assessed by FACS. To confirm MDCK cell expression of β1 integrin related to human β1, we designed a probe corresponding to the C-terminal part of human β1 integrin. This probe directed against the cytoplasmic part of the human β1 integrin was chosen because of the suggested interaction between human CD98 and the cytoplasmic tail of β1 integrin. This specific probe hybridized a 3.6-kb fragment in MDCK cells similar to the hybridized fragment in Caco2-BBE cells (Fig. 6B). Together these results demonstrate that MDCK cells represent a reasonable model to study the effect of expression of human CD98 and its possible interaction with β1 integrin.

MDCK cell line interacts with the canine β1 integrin (Fig. 6C). In contrast, LAT-2 did not immunoprecipitate using our antibody directed against amino acid residues 497–512 of the human LAT-2. These results suggest that: (i) CD98 could be expressed as a monomer in MDCK or (ii) another amino acid transporter with low homology to human LAT-2 is present in MDCK cell line and associates with CD98. Further investigations will be necessary to answer these questions.

The MDCK cell line is able to develop a polarized phenotype and form monolayers when grown on plastic or filters (Fig. 7). Immunofluorescence studies on these cells grown on filters demonstrated the junctional staining of human β1 integrin but not human CD98 (Fig. 7). In addition, MDCK cells display an actin network organization typical to polarized cells (Fig. 7). MDCK cells were transfected with the wild type human CD98 cDNA. We confirmed the expression of human CD98 by Northern blot and FACS analysis (Fig. 6, A and D). The expression of human CD98 in these cells induced complete disorganization of the actin network and loss of lateral staining for β1 integrin (Fig. 7). In addition, the human CD98 was only partially expressed on the membrane with considerable retention in the cytoplasm (Fig. 7). To rule out that the observed effects were due to heterodimerization between CD98 and a canine amino acid transporter, we examined the expression of monomeric human mutated CD98 (C109 was mutated to serine, preventing disulfide linkage between human CD98 and canine amino acid transporter). Interestingly, MDCK cells transfected with the mutated human CD98(C109) presented the same phenotypic alterations as the MDCK cells transfected with the wild type human CD98 (data not shown). These data support the notion that the monomeric form of human CD98 is responsible of the observed phenotype change in MDCK cells.

A Limited Domain of CD98 Appears Necessary and Sufficient to Induce the Phenotype Change—We show that MDCK cells transfected with a truncated mutant human CD98 (D4: deletion of nucleotide acids 1–337, corresponding to amino acids 1–76) also displayed the above described phenotypic change (Fig. 7). In contrast, truncation mutant of human CD98 (D5: deletion of nucleotide acids 1–367, corresponding to amino acids 1–86) did not induce a phenotype change. With this Δ1–367 mutant, β1 integrin and CD98 remained associated with the plasma membrane and the actin network was not disorganized (Fig. 7). The lack of phenotype change was not due to a low expression level of the Δ1–367 CD98 mutant since MDCK cells transfected with the different CD98 constructs demonstrated comparable mRNA and protein expression (Fig. 6, A and D–F). These results suggest that the sequence between amino acids 76 and 86 of CD98 was crucial for the induction of the altered phenotypic change.
DISCUSSION

Here we demonstrate that CD98 is basolaterally polarized in model human intestinal epithelia. In addition, we show that CD98 associates with LAT-2, known to represent an L-type amino acid transporter, forming the heterodimer CD98/LAT-2. Consequently, LAT-2 is also basolaterally polarized. Additionally, β1 integrins, which also polarize basolaterally, associate with CD98 (and likely also CD98/LAT-2 heterodimers). Finally, CD98 can influence β1 integrin distribution and coincidentally cell shape and cytoskeletal order, features known to depend on β1 integrin function, and can do so even in the absence of LAT-2 associations.

As previously described integrins, including β1 integrins, are expressed in the basolateral domain and along cell-cell junctions (lateral domain), where they have a role in maintaining cell-cell adhesion and organization of the subcortical cytoskeleton (18). The fact that β1 integrins co-localize with CD98/LAT-2 to the intercellular contact sites in Caco2-BBE mono-

**FIG. 5.** CD98/LAT-2 co-immunoprecipitate with β1 integrin. Caco2-BBE cell lysates were immunoprecipitated with anti-CD98 (lane 1), anti-β1 integrin (lane 2), no antibody (lane 3), anti-E-cadherin (lane 4), and anti-LAT-2 (lane 5). Immunoprecipitates were subject to 7.5% SDS-polyacrylamide gel electrophoresis, followed by transfer to nitrocellulose membrane. The blot was immunostained with anti-CD98 (A) or anti-LAT-2 (B) antibody.

**FIG. 6.** Expression of different human CD98 constructs in a human CD98-deficient β1 integrin-non-deficient cell line (MDCK). A, Northern blot analysis was performed on total RNA (20 μg) from MDCK (lane 1), MDCK-CD98 (MDCK cells transfected with the wild type human CD98; lane 2), MDCK-D4 (MDCK cells transfected with the deleted CD98, deletion of nucleotide acids 1–337, corresponding to amino acids 1–76; lane 3), and MDCK-D5 (MDCK cells transfected with the deleted CD98, deletion of nucleotide acids 1–367, corresponding to amino acids 1–86; lane 4). Blots were probed with 32P-labeled human CD98 (full-length). A 2.2-kb hybridizing signal corresponding to CD98 was present in MDCK-CD98 (lane 2), MDCK-D4 (lane 3), and MDCK-D5 (lane 4) but not in MDCK wild type (lane 1). The same blot was stripped and re-probed with the GAPDH cDNA. B, Northern blot analysis was performed on total RNA (20 μg) from MDCK (lane 1) and Caco2-BBE (lane 2). Blots were probed with 32P-labeled human β1 integrin (0.30-kb; 3323–3614). A 3.6-kb hybridizing signal corresponding to β1 integrin was present in MDCK (lane 1) and Caco2-BBE (lane 2). The same blot was stripped and re-probed with the GAPDH cDNA. C, MDCK-CD98 cell lysates were immunoprecipitated with anti-CD98 (lane 1), anti-β1 integrin (lane 2), or no antibody (lane 3). Immunoprecipitates were subject to 7.5% SDS-polyacrylamide gel electrophoresis followed by transfer to nitrocellulose membrane. The blot was immunostained with anti-CD98. Flow cytometric analysis of CD98 on MDCK-CD98 (D), MDCK-D4 (E), and MDCK D5 (F) cells in absence (−AB) or in presence (+AB) of mouse anti-CD98 antibody.
layers suggests that CD98 may also regulate $\beta_1$ integrin function. Given the importance of integrin cytoplasmic tails in integrin activation, proteins such as CD98 that interact with integrin cytoplasmic domains are potentially excellent candidates as modifiers of integrin activation. Integrins are dynamic molecules, and recent studies have either suggested or demonstrated that a number of surface transmembrane glycoproteins can associate with integrins and, in doing so, modulate their function (19–23). Several classes of cell surface glycoproteins have been shown to play a role in integrin-mediated events including the integrin-associated protein (CD47) and transmembrane 4 superfamily (TM4SF or tetraspanins). CD47 associates with the $\alpha$-$\beta_1$ integrin and appears to influence integrin-mediated signal transduction, phagocytosis, and cell migration (24–27). Numerous TM4SF members such as CD36, CD63, and CD9 have been shown to be associated with $\beta_1$ integrins (20–23). Recently, oocyte experiments (15) showed that the amino acid transporter LAT-2 could be influenced by CD98 (15) and, in non-polarized CHO cells it was shown that CD98 could modify the function of $\beta_1$ splice variants expressed by epithelia (11). Together, such observations raise the possibility that CD98, if basolaterally expressed by polarized epithelia, could influence both classic transport and attachment/adherence/integrin-mediated cytoskeletal functions.

Epithelial cell polarity is determined by a combination of events mediated by cell-cell and cell-substratum adhesion. Cell adhesion to the ECM is mediated by the integrin superfamily of adhesion receptors and is thought to play a critical role in subsequent ordering of the cytoskeleton and formation of polarity. However, the interactions between integrins and ECM, although triggering a crude initial polarity, are unlikely to be sufficient to organize the refined polarity displayed by polarized columnar epithelia. Additional cell-cell interactions also are likely required for the latter event in order to restrict the localization of basolateral proteins and achieve a columnar stature. In the present study, we have shown that the expression of human heterodimeric (human CD98/endogenous canine light chain) or monomeric human CD98 in a CD98-deficient cell line (MDCK) induces the disruption of intercellular adhesion and eventuates in cytoskeletal disorder. This phenotypic conversion, which probably depends on the interaction of human CD98 with respective "ligands," is accompanied by the reorganization of the actin cytoskeleton. The phenotypic conversion did not involve CD98 effects on the endogenous canine amino acid transporter, as it was also observed with the overexpression of CD98 modified at a specific residue that prevents such associations. In contrast, recently it has been demonstrated that heterodimeric CD98, but not monomeric CD98, causes transformation of fibroblasts cells, but here the expression of the amino acid transporter was thought essential to achieve this phenotype (28). A potential ligand for CD98 is $\beta_1$ integrin, which is expressed by MDCK cells. The observed phenotypic conversion observed with CD98 could be related to altered $\beta_1$ recognition of extracellular ligands (10), and effects of CD98 on $\beta_1$ integrin function have been demonstrated (10–12, 29). Consistent with this view, overexpression of CD98 with the cytoplasmic tail and part of the transmembrane domain deleted did not induce this phenotypic change, whereas a partial truncation variant of the cytoplasmic tail of CD98 retained the phenotypic change. These results suggest that a cytoplasmic juxtaplasmic domain and/or intramembrane domain is crucial in this phenotype change.

In conclusion, we have demonstrated that the heterodimer CD98/LAT-2 is specifically expressed in the basolateral membrane of Caco2-BBE monolayers. The co-localization of CD98/ LAT-2 and $\beta_1$ integrin suggests a possible interaction between these three proteins. We speculate that specific molecular ratio between the heterodimer CD98/LAT-2 and $\beta_1$ integrin may be required for the polarity of epithelial cells. Expression of human CD98 in a human CD98-deficient cell line (MDCK) may change the molecular ratio of the canine CD98/canine amino acid transporter and $\beta_1$ integrin with consequent effect on cell adhesion and polarity. The cytoplasmic juxtaplasmic domain and/or intramembrane domain appears crucial in this process.

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