We have previously shown that the heterodimer CD98/LAT-2 (LAT-2: amino acid transporter) is expressed in the basolateral membrane of intestinal epithelia and is associated with $\beta_1$ integrin (Merlin, D., Sitaraman, S., Liu, X., Easterburn, K., Sun, J., Kucharzik, T., Lewis, B., and Madara, J. L. (2001) J. Biol. Chem. 276, 39282–39289). In the present study we examined the interaction of CD98/LAT2 with intracellular adhesion molecule I (ICAM-1) and the potential of such interaction on the activation of intracellular signal in Caco2-BBE cell monolayers. ICAM-1 was found to be expressed to the basolateral domain and to selectively communoprecipitate with CD98/LAT-2 in Caco2-BBE monolayers. Using antibodies as ligands to CD98 and ICAM-1, we demonstrate that the basolateral cross-linking of CD98 and ICAM-1 specifically affects the intrinsic activity of the LAT-2 transporter. Whereas CD98 ligation decreases the $K_m$ and $V_{\text{max}}$ of the LAT-2 transporter, ICAM-1 ligation increases $K_m$ and $V_{\text{max}}$ of the amino acid transporter LAT-2. In addition, basolateral cross-linking of CD98 or ICAM-1 induces threonine phosphorylation of an ~160-kDa supramolecular complex that is consistent with CD98/LAT-2-ICAM-1 complex. Together these findings demonstrate that (i) CD98/LAT2 interacts with ICAM-1 in Caco2-BBE cell monolayers, and (ii) CD98 and ICAM-1 ligands generate intracellular signals that regulate the amino acids transporter (LAT-2) activity. Our data provide a novel mechanism by which events such as adhesion could be integrated by amino acid transport activity resulting from the direct interaction of cell surface molecules such as CD98 and ICAM-1.

Glycoprotein CD98 is a cell surface heterodimer formed by the covalent linkage of CD98 heavy chain with several different light chains to form amino acid transporters (1). Recently it has been demonstrated that the heterodimer CD98/LAT-2 is found only in tissues containing epithelial barriers (2, 3). The amino acid transporter LAT-2, a protein of 555 amino acids, is highly expressed in polarized epithelia (4, 5). In absorptive epithelia of the small intestine and in the small intestine-like cell line Caco2-BBE monolayers, the heterodimer CD98/LAT-2 is polarized to the basolateral side (3).

CD98 and Intracellular Adhesion Molecule I Regulate the Activity of Amino Acid Transporter LAT-2 in Polarized Intestinal Epithelia*

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There is a growing literature implicating CD98 in integrin function (6–8). Recently we have demonstrated (9) that $\beta_1$ integrins, which are also polarized basolaterally in intestinal epithelial cells, associate with CD98/LAT-2. We found that CD98 not only influences $\beta_1$ integrin distribution but also affects the cell shape and cytoskeletal order, features known to depend on $\beta_1$ integrin function (10). Interestingly, CD98 interacts specifically with $\beta_1$ integrins but not with the muscle-specific splice variant $\beta_{1D}$ or the leukocyte-specific $\beta_2$ integrin (6). The basolateral location of CD98 suggests that this protein could be involved in some form of cell signaling where binding of ligand to the extracellular loop of CD98 results in an alteration in cellular function via the regulation of the amino acid transporter (LAT-2 in the intestine) and $\beta_1$ integrin functions (9). At present, possible ligands for CD98 remain to be determined, but it was reported that galectin-3, a 26-kDa $\beta$-galactosidase binding protein of the galectin family (10, 11), could bind to CD98 on T cells. It is possible that CD98 activation could regulate the activity of the amino acid transporter LAT-2.

Because epithelial cells rest on the extracellular matrix (ECM), it is logical to expect specific interactions between basolateral “receptors” such as CD98 or ICAM-1 and ECM. Indeed, upon binding to ECM ligands (outside), integrins deliver signals that control cell proliferation, gene induction, differentiation, and proliferation. The basolateral location of the heterodimer CD98/LAT-2 suggests that CD98 may interact with other adhesion molecules. Among adhesion proteins, the intercellular adhesion molecule ICAM-1 has been shown to be expressed in inflamed epithelial cells (12, 13). ICAM-1 is known to be the receptor to the heterodimer of CD11a, and CD18 ($\beta_2$ integrin) is expressed in leukocytes. It is conceivable that in the intestinal epithelia, ICAM-1 may be part of a multicomponent web that includes CD98/LAT2 and integrin $\beta_1$. The multicomponent web could orchestrate epithelial cell function such as LAT-2-mediated amino acid transport activity. We hypothesized that the amino acid transporter LAT-2 may be regulated by adhesion molecules such as ICAM-1 and CD98 in epithelial cells. In the present study we investigate (i) the expression of ICAM-1 in intestinal epithelial cell line Caco2-BBE, (ii) the possibility that ICAM-1 interacts with CD98/LAT-2, and (iii) the effects of cross-linking ICAM-1 and CD98 on LAT-2-mediated amino acid transport activity.

MATERIALS AND METHODS

Cell Culture—Caco2-BBE (14–16) cells were grown as confluent monolayers in a 1:1 mixture of Dulbecco's Vogt-modified Eagle's media and Ham's F-12 medium supplemented with 15 mM HEPES buffer (pH 7.5), 14 mM NaHCO3, and 10% new-born calf serum. Monolayers were subcultured every 7 days by trypsinization with 0.1% trypsin and 0.9 mM EDTA in Ca2+/Mg2+-free phosphate-buffered saline. Cell surface biotinylation and cross-linking studies were carried out with confluent

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† The abbreviation used is: ICAM, intracellular adhesion molecule.

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monolayers plated on collagen-coated permeable supports (area 0.3 cm², pore size 0.4 μm) and examined 10 days post-plating.

**RT-PCR of ICAM-1 Expression**—The expression of ICAM-1 in Caco2-BBE cells was determined using an RT-PCR method with oligonucleotide primers specific for ICAM-1. Total RNA was isolated from confluent Caco2-BBE cells cultured on plastic supports (area: 9.4 cm²) for 14 days with a Micro Fast Track™ kit (Invitrogen). The yield of RNA from each preparation was determined by ultraviolet spectrophotometry. 1 μg of total RNA was primed with oligo(dT) and reverse-transcribed with avian myeloblastosis virus-reverse transcriptase (cDNA cycle kit; Invitrogen). A dilution of the reverse transcription reaction was used as a template for amplification by PCR. After an initial denaturation at 94 °C for 5 min, PCR of the samples was carried out for 55 cycles under the following conditions: denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 3 min. This was followed by a final extension step at 72 °C for 7 min. For detection of ICAM-1, the primers specific for ICAM-1 corresponded to nucleotide positions 53–70 (sense: 5'-TCGCTATGGCTCCCAGCA-3') and 1662–1645 (antisense: 5'-ATAGGTTACGAGGGGG-3') of the cDNA (GenBank™ GL:220-51567) were used that yield a product of 1,592 bp. PCR products were separated by electrophoresis on 1% agarose gels, visualized by ethidium bromide, and verified by DNA sequencing.

**Cell Surface Biotinylation**—Filter-grown cells were rinsed twice with phosphate-buffered saline supplemented with 0.1 mM CaCl₂ and 1 mM MgCl₂. Basolateral or apical sides of the monolayers were incubated with freshly prepared sulfosuccinimidobiotin (s-NHS-biotine; Pierce) for 24 h at 4 °C. Subsequently, 50 μl of protein G-agarose suspension (50 μl of beads). The beads were pelleted by centrifugation at 12,000 × g for 20 s in a microfuge. Supernatants were transferred to fresh tubes, and the appropriate amount of specific antibody (1:1000 dilution of goat anti-CD98 (RDI), sheep anti-CD98) 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 × g for 20 s by microfuge. The beads were washed two times for 20 min with buffer 1 (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P40), buffer 2 (50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 1 mM EDTA, 1% Nonidet P40), and buffer 3 (10 mM Tris-HCl, pH 7.5, 0.1% Nonidet P40). 50 μl of gel loading buffer (1% (w/v) Triton X-100 in 20 mM Tris, pH 8.0, 50 mM NaCl, 5 mM EDTA, 2% SDS, and 0.2% (w/v) bovine serum albumin supplemented with protease inhibitors 2% SDS) was added to the agarose pellet and 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 goat anti-CD98 (RDI), sheep anti-ICAM-1. They were further incubated for 30 min at room temperature with the appropriate horseradish peroxidase-conjugated antibody diluted 1:1000 and probed using ECL (Amer sham Biosciences).

**Cross-linking CD98 and ICAM-1**—Caco2-BBE cells were grown to confluence on permeable supports (area: 0.6 cm², pore size: 0.4 μm). Before the experiment, Caco2-BBE monolayers were washed three times with buffer containing 100 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, and 10 mM HEPES (pH 7.4) at room temperature. Caco2-BBE monolayers were incubated apically with 200 μl of incubation medium and basolaterally with 500 μl of incubation medium containing 5, 10, or 20 μg/ml CD98 or ICAM-1 antibody with the appropriate secondary antibody (1:1000 dilution) or with only the secondary antibody (1:1000 dilution) for 1 h at 37 °C.

**Amino Acid Transport Uptake Assay**—We investigated the effect of cross-linking CD98 or ICAM-1 on amino acid (leucine) transport across basolateral membranes of Caco2-BBE monolayers. Cells grown on filters were washed twice with a buffer containing 100 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, and 10 mM HEPES, pH 7.4. After CD98 or ICAM-1 cross-linking (see above), Caco2-BBE monolayers were transferred in new wells in Na⁺-free buffer containing [3H]leucine at different concentrations, 100 mM choline chloride, 2 mM KCl, 1 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P40) containing 1 mg/ml aprotinin, 1 mg pepstatin, 2 mM serine proteases. The lysates were centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting 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 × g for 20 s in a microfuge. Supernatants were transferred to fresh tubes, and the appropriate amount of specific antibody (1:1000 dilution of goat anti-CD98 (RDI), sheep anti-ICAM-1) 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 × g for 20 s by microfuge. The beads were washed two times for 20 min with buffer 1 (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P40), buffer 2 (50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 1 mM EDTA, 1% Nonidet P40), and buffer 3 (10 mM Tris-HCl, pH 7.5, 0.1% Nonidet P40). 50 μl of gel loading buffer (1% (w/v) Triton X-100 in 20 mM Tris, pH 8.0, 50 mM NaCl, 5 mM EDTA, 2% SDS, and 0.2% (w/v) bovine serum albumin supplemented with protease inhibitors 2% SDS) was added to the agarose pellet and 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 goat anti-CD98 (RDI), sheep anti-ICAM-1. They were further incubated for 30 min at room temperature with the appropriate horse radish peroxidase-conjugated antibody diluted 1:1000 and probed using ECL (Amersham Biosciences).
radioactivity of sample was determined by liquid scintillation.

37°bation medium was taken from the basolateral reservoir after 2 min at pH 9.26 reservoir. To measure basolateral leucine effluxes, 500 μL of incubation medium (Na

MgCl₂, 1 mM CaCl₂, 10 mM HEPES, pH 7.2) to the basolateral reservoir. Uptakes were performed for 2 min at 37°C, washing each filter in buffer solution at 4°C stopped the reaction. The radioactivity of each filter was determined by liquid scintillation.

Amino Acid Efflux Assay—We investigated the effect of cross-linking CD98 or ICAM-1 on amino acid (leucine) efflux across basolateral membranes of Caco2-BBE monolayers. Cells grown on filters were washed twice with a buffer containing 100 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 10 mM HEPES, pH 7.2 to the apical reservoir. Uptakes were performed for 2 min at 37°C; washing each filter in buffer solution at 4°C stopped the reaction. The radioactivity of each filter was determined by liquid scintillation.

Expression of ICAM-1 in Caco2-BBE Monolayers—Expression of ICAM-1 mRNA was assessed by RT-PCR in Caco2-BBE cells. Primers were used that yielded a 1.6-kb product. The PCR product was purified from 1% agarose gel using a DNA extraction kit (Qiagen Inc.), and the sequence showed 100% homology sequence to the published coding sequence of ICAM-1 (bases 53–1662). Additionally, ICAM-1 was detected at the protein level by Western blotting in Caco2-BBE cells (Fig. 1B). Using the anti-human ICAM-1 antibody, Caco2-BBE cell lysates displayed a single immunoreactive band corresponding to ~90 kDa (Fig. 1B, lane 1) that was comparable with the immunoreactive band from the recombinant ICAM-1 (Fig. 1B, lane 2).

ICAM-1 Is Expressed at Both Apical and Basolateral Membranes of Caco2-BBE Monolayers—The membrane localization of the human ICAM-1 was assessed in confluent Caco2-BBE monolayers. We examined the plasma membrane expression of ICAM-1 by surface biotinylation. 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-ICAM-1 antibody (see Materials and Methods) was performed in Na⁺'-free buffer at pH 6.2, the optimal conditions for LAT-2-mediated amino acid uptake. To examine whether CD98 or ICAM-1 ligation affects leucine uptake by modifying the intrinsic activity of the amino acid transporter, the effect of basolateral CD98 or ICAM-1 ligation on the kinetics of [³H]leucine uptake was studied. Kinetics analysis of the data (Fig. 3) indicated that CD98 ligation significantly decreased the Vₘₐₓ
Fig. 4. The effects of cross-linking ICAM-1 on leucine uptake LAT-2-mediated transport across basolateral membrane in Caco2-BBE monolayers. The concentration dependence of L-leucine uptake for 2 min was determined in the Na+-free uptake solution at pH 6.20 as described under “Materials and Methods.” The concentration dependence profile of leucine transport was shifted by cross-linking ICAM-1. The values were fitted to the Michaelis-Menten curve. 20 μg/ml of the ICAM-1 antibody with the appropriate secondary antibody (1:1000 dilution) (+anti-ICAM-1) or only the secondary antibody (1:1000 dilution) (–anti-ICAM-1) were added to the basolateral aspect of Caco2-BBE monolayers for 1 h at 37°C as described under “Materials and Methods.” All data are the mean of three independent experiments performed in triplicate ± S.D.

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(V_{\text{max(control)}} = 1256 + 160 \text{ versus } V_{\text{max(CD98 ligation)}} = 576 + 47.7 \text{ nmol/mg protein/2 min}) \text{ and the } K_m \text{ of the transporter } (K_m(\text{control}) = 7.45 + 1.86 \text{ versus } 3.01 + 0.69 \text{ mM}). \text{ In contrast, as shown in Fig. 4 ICAM-1 ligation significantly increased the } V_{\text{max(CD98 ligation)}} = 1306 + 336 \text{ versus } V_{\text{max(ICAM-1 ligation)}} = 3504 + 1200 \text{ nmol/mg protein/2 min}) \text{ and the } K_m \text{ of the transporter } (K_m(\text{control}) = 7.65 + 3.80 \text{ versus } 24.8 + 11.3 \text{ mM}). \text{ Together these data demonstrate that CD98 and ICAM-1 ligation differently affects the intrinsic activity of the amino acid transporter in Caco2-BBE monolayers.}

Amino Acid Efflux across Basolateral Membranes of Caco2-BBE Is Regulated by CD98 and ICAM-1 Ligations—The efflux of radioactivity across basolateral membranes of the Caco2-BBE monolayers preloaded with [14C]leucine was measured in the absence of extracellular L-leucine in the extracellular medium (see “Materials and Methods”). Adding L-leucine into the extracellular medium did not significantly increase the L-[14C]leucine efflux (data not shown) that is one of the characteristics of LAT-2 amino acid transporter. Fig. 5, A and B shows that cross-linking basolateral CD98 and cross-linking basolateral ICAM-1 in Caco2-BBE monolayers induced a concentration-dependent increase of [3H]leucine efflux when compared with the controls (basolateral membranes of Caco2-BBE were incubated with only the appropriate secondary antibody (1:1000 dilution)). The increase of [3H]leucine efflux began to occur significantly at 5 μg/ml CD98 or ICAM-1 antibody concentration (anti-CD98:14.2% increase 0.3883 + 0.0130 versus 0.4435 + 0.0367 nmol/mg protein/2 min; anti-ICAM-1: 32.0% increase 0.4631 + 0.0114 versus 0.6111 + 0.0672 nmol/mg protein/2 min). At 10 μg/ml CD98 antibody or ICAM-1 antibody concentration, the [3H]leucine efflux across basolateral membranes of Caco2-BBE monolayers was 24.0 and 44.0%, respectively (anti-CD98: 0.3883 + 0.0130 versus 0.4824 + 0.0543 nmol/mg protein/2 min; anti-ICAM-1: 0.4631 + 0.0114 versus 0.6693 + 0.0646 nmol/mg protein/2 min), and achieved a 27.4.7% increase at 20 μg/ml CD98 antibody (0.3883 + 0.0130 versus 0.4950 + 0.040 nmol/mg protein/2 min) and a 58.8% increase at 20 μg/ml ICAM-1 antibody (0.4631 + 0.0114 versus 0.7321 + 0.0451 nmol/mg protein/2 min). These results demonstrate that CD98 and ICAM-1 may be important for controlling the LAT-2-mediated amino acid efflux across basolateral membranes of Caco2-BBE monolayers.

Basolateral CD98 and ICAM-1 Ligations Induce Same Pattern of Threonine Phosphorylation of a 160-kDa Protein in Caco2-BBE Monolayers—Basolateral CD98 and ICAM-1 ligations in Caco2-BBE monolayers were performed for 1 h at 37°C as described under “Materials and Methods.” Caco2-BBE cell lysates were subjected to immunoprecipitation for CD98 or ICAM-1. ICAM-1 and CD98 immunoprecipitates were detected by anti-phosphothreonine antibody. The CD98 (Fig. 6, lane 1) and ICAM-1 (lane 5) immunoprecipitates display an ~160-kDa band that was induced by basolateral CD98 ligation (CD98 control ligation; Fig. 6, lanes 2 and 6). In addition, ICAM-1 (Fig. 6, lane 7) immunoprecipitate displays the same induced 160-kDa band after ICAM-1 ligation (control ICAM-1 ligation; Fig. 6, lane 8). In contrast, CD98 (Fig. 6, lane 3) immunoprecipitate did not display the 160-kDa band after ICAM-1 ligation (control ICAM-1 ligation; Fig. 6, lane 4), suggesting that ICAM-1 ligation induced a less potent protein phosphorylation than CD98 ligation. The detected phosphorylated protein at 160 kDa is likely to be CD98 or one of the molecules complexed to CD98 that immunoprecipitates at ~160 kDa.

**DISCUSSION**

We have demonstrated that ICAM-1 is constitutively expressed on basolateral and apical membranes of Caco2-BBE monolayers. These results are in agreement with studies showing that the well differentiated Caco2-BBE cell line shows the highest constitutive expression of ICAM-1 when compared with other less differentiated cell lines such as T84 or HT29 (12). The expression of ICAM-1 in the basolateral aspect of Caco2-BBE monolayers makes these cells an appropriate cellular model for study of the interaction of ICAM-1 with other basolaterally expressed proteins.

ICAM-1 is a cell adhesion molecule that plays an important role in cell-cell, cell-extracellular matrix interactions and cellular interactions such as the immune response (17). In the present study, we have demonstrated that ICAM-1 associates with the heterodimer CD98/LAT2. This result suggests that to accomplish a task such as adhesion ICAM-1 works not only as an individual receptor but also as a component of supramolecular complexes at the plasma membrane in epithelial cells. In addition, the association of the heterodimer CD98/LAT2-2 and ICAM-1 suggest that there may be significant cellular regulation mediated by this supramolecular complex. The complex may induce signals, via the amino acid transporter LAT-2, to regulate multiple aspects of cell physiology. For example, regulation of intracellular amino acid availability mediated by LAT-2 transport activity may modulate the activity-signaling pathway, which leads to phosphorylation of an intracellular target protein. In addition, it has been demonstrated that the intracellular amino acid supply modulates several important...
regulatory translation factors through a variety of mechanisms (18). Furthermore, it has been shown that amino acid leucine availability regulates the activity of the signaling pathway, which leads to the activation of p70 S6 kinase (the 70-kDa protein kinase acting on ribosomal protein S6) (19, 20). In the present study, we report that cross-linking CD98 or ICAM-1, which somehow mimics natural ligands for these proteins, modifies leucine LAT-2-mediated transport activity. Interestingly, cross-linking CD98 and ICAM-1 differentially affects the LAT-2 transport activity.

CD98 disulfide linked to LAT-2 is basolaterally expressed in intestinal epithelia and in Caco2-BBE (3); the resulting dimer is the minimal functional unit for a Na⁺/H⁺-independent transporter for zwitterionic amino acids. The extracellular domain of CD98 is responsible for recognition of LAT-2, and that extracellular domain ensures proper translocation to the plasma membrane (1). The mode of regulation of amino acid transport by both subunits (CD98 and LAT-2) and the possible interplay between them remains largely unstudied. In the present study, we show that cross-linking CD98 affects the intrinsic activity of the LAT-2 transporter by increasing the affinity and reducing the capacity of LAT-2-mediated uptake of leucine. In addition, we demonstrate that cross-linking CD98 regulates the LAT-2-dependent leucine efflux. However, it will be of interest to characterize the transport kinetics of LAT-2 for the export of amino acids to know whether this amino transporter shows an asymmetric amino acid transport function. Interestingly, we demonstrate that cross-linking ICAM-1 decreases the affinity and increases the capacity of LAT-2-mediated leucine uptake. Furthermore, cross-linking of ICAM-1 increases leucine efflux across the basolateral membranes of Caco2-BBE.

We suggest that the transport activity changes are the result of a direct or indirect phosphorylation of the LAT-2 transporter. Cross-linking CD98 or ICAM-1 induces threonine phosphorylation of a 160-kDa protein complex. Basolateral cross-linking CD98 (CD98 ligation) or ICAM-1 (ICAM-1 ligation) at 20 μg/ml in Caco2-BBE monolayers was performed for 1 h as described under "Materials and Methods." As a ligation control, secondary antibody against CD98 or ICAM-1 was incubated for 1 h (CTRL CD98 ligation, CTRL ICAM-1 ligation). Caco2-BBE cell lysates were immunoprecipitated with anti-CD98 (IP CD98), anti-ICAM-1 (IP ICAM-1). Immunoprecipitates were subjected to 10% SDS-polyacrylamide gel electrophoresis, followed by transfer to nitrocellulose membrane. CD98 and ICAM-1 immunoprecipitates were immunoblotted using anti-phosphothreonine antibody. This blot is representative of five blots with identical patterns.

In conclusion, the amino acid transporter LAT-2 is regulated by adhesion molecules such as ICAM-1 and CD98 in epithelial cells. CD98 and ICAM-1 may play a role in delivering intracellular signals. Changes in amino acid transport activity resulting from CD98 and ICAM interaction may ensure integration of events such as cell adhesion.
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