Distinct Domains of CD98hc Regulate Integrins and Amino Acid Transport*

CD98 is a cell surface heterodimer formed by the covalent linkage of CD98 heavy chain (CD98hc) with several different light chains to form amino acid transporters. CD98hc also binds specifically to the integrin $\beta_{1A}$ cytoplasmic domain and regulates integrin function. In this study, we examined the relationship between the ability of CD98hc to stimulate amino acid transport and to affect integrin function. By constructing chimeras with CD98hc and a type II transmembrane protein (CD69), we found that the cytoplasmic and transmembrane domains of CD98hc are required for its effects on integrin function, while the extracellular domain is required for stimulation of isoleucine transport. Consequently, the capacity to promote amino acid transport is not required for CD98hc’s effect on integrin function. Furthermore, a mutant of CD98hc that lacks its integrin binding site can still promote increased isoleucine transport. Thus, these two functions of CD98hc are separable and require distinct domains of the protein.

CD98hc is a widely distributed transmembrane protein that was originally discovered as a T-cell activation antigen (1). CD98hc expression is tightly linked to cell proliferation, and antibodies against CD98hc can inhibit cell growth or induce apoptosis in specific cell types (2, 3). A compelling body of evidence implicates CD98hc in the transport of amino acids. CD98hc overexpression stimulates multiple amino acid transport systems including L, y+,L, and x~ (4). Furthermore, mutations in its closest parologue, D2 (r-BAT), lead to a disorder hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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These light chains have multiple membrane-spanning domains, they resemble permeases and are believed to provide the amino acid transport activity of CD98 (6–8). CD98hc may act to regulate the expression and cellular localization of the amino acid transporting activity of the light chain (6, 9). Thus, this widely distributed membrane protein is strongly implicated in amino acid transport.

There is also a growing literature implicating CD98hc in integrin function. Integrins are heterodimeric adhesion receptors expressed in almost every multicellular animal cell type (10). Cells can rapidly modulate their integrins’ affinity for extracellular ligands (activation), thereby regulating multiple integrin-dependent functions (11). Integrin activation is inhibited by overexpression of isolated $\beta_{1A}$ integrin cytoplasmic domains (dominant suppression) (12). CD98hc was identified as an integrin regulator in an expression-cloning scheme for proteins that can complement dominant suppression (COSD)1 (13). In addition, CD98hc binds to integrin $\beta_{1A}$ cytoplasmic domains, and this interaction correlates with COSD (14). Furthermore, clustering CD98hc activates multiple integrin-dependent functions (13, 15, 16) and mimics $\beta_{1A}$ integrin cosignaling in T-cells (17). Thus, CD98hc physically and functionally interacts with integrin adhesion receptors. Indeed, clustering of CD98hc can stimulate several classes of integrins in multiple cell types (13, 15, 16).

In the present study, we have assessed the relationship between the amino acid transport and integrin regulatory activities of CD98hc. We found that the CD98hc alone is necessary and sufficient for the interaction of CD98 with the $\beta_{1A}$ integrin tail and for COSD. By forming chimeras between CD98hc and another type II transmembrane protein, we found that the cytoplasmic and transmembrane domains of CD98hc are required for integrin interactions but not for stimulation of isoleucine transport. In contrast, the CD98hc extracellular domain was required for the stimulation of amino acid transport. Thus, the amino acid transport activity and integrin interactions of CD98 are independent activities of the protein and are mediated by different domains of CD98hc.

**EXPERIMENTAL PROCEDURES**

Antibodies—The hybridoma cell line 4F2(C13) (anti-CD98hc) was purchased from American Type Culture Collection (ATCC, Manassas, VA). The CD98hc antibody was purified from ascites produced in pristane-primed BALB/c mice by protein A affinity chromatography. Dr. S. Shattil (Scripps Research Institute) generously provided the activation-specific anti-CD11b monoclonal antibody, PAC1 (18). The anti-

1 The abbreviations used are: COSD, complement dominant suppression; CHO, Chinese hamster ovary; Pipes, 1,4-piperazinedinediethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; AI, activation index; HBSS, Hanks’ buffered salt solution.
ti-αβ3-activating monoclonal antibody, anti-LIBS6, has been described previously (19). The anti-Tac antibody, 7G7B6, was obtained from the ATCC and was biotinylated with biotin-N-hydroxysuccinimide (Sigma) according to manufacturer’s instructions. The α3β1-specific peptide inhibitor, Ro43-5054, was a generous gift from B. Steiner (Hoffmann-La Roche, Basel, Switzerland). The mouse hybridoma cell line, 12C5 (anti-HA), was purchased from ATCC. The anti-CD98 antibody (C1, a rabbit polyclonal antibody) was a generous gift from Drs. F. Diaz-Gonzalez and F. Sanchez-Madrid (University of Madrid, Spain).

DNA Constructs and Recombinant Proteins—cDNAs encoding the expressed integral cytoplasmic domains joined to four heptad repeats were cloned into the modified pET-15 vector described previously (21). Recombinant expression in BL21 (DE3) pLysS cells (Novagen) and purification of the recombinant model proteins were performed in accordance with the manufacturer’s instructions (Novagen), with an additional final purification step on a reverse phase C18 high performance liquid chromatography column (Vydac). Polyepitide masses were confirmed by electrospray ionization mass spectrometry on an API-III quadrupole spectrometer (Sciex; Toronto, Ontario, Canada), and they varied by less than 4 daltons from those predicted by the desired sequence.

Tac-α5 and Tac-β1A DNA in modified CMV-IL2R expression vectors (22) were generously provided by Drs. S. LaFlamme and K. Yamada (National Institutes of Health). Human 4F2 antigen (CD98hc) cDNA was kindly provided by M. Leiden (University of Chicago, Chicago, IL) and was subcloned into pcDNA1 and EcoRI fragment (C109S, C330S), C1 (C109S), and C2 (C330S) mutants of CD98hc were gifts from Dr. M. Palacin (Universitat de Barcelona). cDNA encoding amino acids amino acids 1–81 and 105–529 of CD98hc and amino acids 39–199 of CD69.

Stimulation of CD98hc using soluble recombinant Tac-α5 (as binding protein) and in vivo-generated antibodies has been quantified as an activation index (AI) defined as (F−F F) (FLIBS6−F) in which F is the median fluorescence intensity of PAC binding, F, is the median fluorescence intensity of PAC binding in the presence of saturating concentration of a competitive inhibitor (1 μM Ro43-5054), and FLIBS6 is the maximal median fluorescence intensity in the presence of the integrin activating antibody, anti-LIBS6 (2 μM). Percent reversal was calculated as [AI(α5)] − [AI(α5)]/[AI(α5)] and [AI(α5)] AI(α5) is the AI of cells transfected with Tac-β1A chimeras, [AI(α5)] is the AI of cells cotransfected with CD98hc and Tac-β1A chimeras, and [AI(α5)] is the AI of cells transfected with Tac-α5.

Amino Acid Transport—H-Labeled Ile (77 Cl/mM) was purchased from Amersham Pharmacia Biotech. αβ3cys were transfected with the C330S (Cless) mutation, is reported to reduce the amino acid transport activity of CD98 (7, 25, 26). However, the capacity of CD98hc to complement dominant suppression was not impaired by mutation of both or either cysteines (Fig. 1A). We confirmed that the mutant lacking cysteines (Cless) failed to form stable disulfide-bonded heterodimers with CD98 light chain.

RESULTS

CD98 Heavy Light Chain Association Is Not Required for the Interaction of CD98 with Integrins—CD98 is a heterodimer formed by a common heavy chain (CD98hc) disulfide-bonded to one of a number of light chains that mediate amino acid transport. CD98hc has two extracellular cysteines, one of which (Cys180) is involved in covalent association of the heavy and light chains (25). To examine the role of CD98 heavy-light chain association on its interactions with integrins, we first investigated the effect of mutation of these cysteines (Cys180 and Cys309). The C109S (Cout) single mutation, or in combination with the C330S (Cless) mutation, is reported to reduce the amino acid transport activity of CD98 (7, 25, 26). However, the capacity of CD98hc to complement dominant suppression was not impaired by mutation of both or either cysteines (Fig. 1A). We confirmed that the mutant lacking cysteines (Cless) failed to form stable disulfide-bonded heterodimers with CD98 light.
The interaction of CD98hc with the integrin β2 domain.

Figure 1: Stable formation of the CD98 heterodimer is not required for its effects on integrin function. A, effect of cysteine mutations on CODS. αβPy cells were transfected with Tac-β1 and either wild-type CD98hc or CD98hc with serine substitutions at cysteines (Cys109, Cys 330) involved in covalent heterodimer formation. Twenty-four h after transfection, cells were collected and the Tac-positive subset of cells was analyzed for the ability to bind to the PAC1 antibody. Data are expressed as percentage reversal of Tac-β1 suppression, which is calculated as 100 × (ΔAItransp1 + CD98hc – ΔAItransp1/ΔAItransp1 – ΔAItransp1/mock). AI is the activation index of cells transfected with Tac-β1 alone or in combination with CD98hc or with Tac-α, Cless = CD98hc (C109S,C330S), C1 = CD98hc (C109S), C2 = CD98hc (C330S). B, CD98hc Cys109 and Cys330 are not required for binding to the integrin β1A cytoplasmic domain. αβPy cells were transfected with either CD98hc, the Cless mutant, or vector DNA. After 24 h surface proteins were labeled with Sulfo-Biotin N-hydroxysuccinimide, cell lysates were incubated with beads coated with model proteins containing β1A or αm tails. The bound proteins were eluted and immunoprecipitated with anti-CD98hc and fractionated by reduced SDS-PAGE. Biotinylated CD98hc was identified by streptavidin-peroxidase-generated chemiluminescence staining of an ~80-kDa polypeptide. In addition, the starting cell lysate was immunoprecipitated with either anti-CD98hc antibodies (Lysate) or with a control IgG (IgG). Proteins were fractionated by reduced SDS-PAGE, and biotinylated proteins were detected by streptavidin-peroxidase-generated chemiluminescence. C, mutation of CD98hc cysteines 109 and 130 disrupts covalent heterodimer formation. Biotinylated lysates of surface labeled cells transfected with either CD98hc or Cless CD98hc were immunoprecipitated with anti-CD98hc antibodies as described in B. The lysates were fractionated by SDS-PAGE in the absence or presence (+DTT) of reducing agent. Biotinylated polypeptides were identified by streptavidin-peroxidase-generated chemiluminescence.

As an alternative approach to evaluate the role of the CD98 heavy-light chain association, we examined the effect of increased expression of a CD98 light chain (hLAT1) on integrin interactions. When CHO cells were transfected with CD98hc, there was a substantial quantity of free heavy chain (Fig. 2B). Transfection of increasing quantities of a light chain, hLAT1, resulted in an increasing proportion of CD98 heterodimers (Fig. 2B). As expected, formation of increased CD98 heterodimers led to a marked increase in amino acid transport (Fig. 2A). However, increased heterodimer formation did not detectably alter the ability of CD98hc to bind to β1A cytoplasmic tails (Fig. 2C). Thus, while the formation of CD98 heterodimers is important for the stimulation of amino acid transport, CD98hc alone is sufficient for binding to the integrin β1A tail and for CODS.

The NH2 Terminus of CD98hc Is Intracellular, and the...
In the foregoing experiments, we found that the interactions of CD98 with integrins could be ascribed to its heavy chain. Consequently, we wished to analyze the structural determinants in the heavy chain responsible for these interactions. CD98hc is predicted to be a type II transmembrane protein, with the COOH terminus extracellular and the NH2 terminus intracellular. To document the membrane topography of CD98hc, the NH2 and COOH terminus of CD98hc were separately tagged using a HA-tag. When cells were transfected with the COOH-terminal-tagged cDNA, the epitope tag was readily detected on the cell surface as indicated by a rightward shift in the fluorescence intensity (Fig. 3A). In contrast, the amino-terminal tag was not detected on the cell surface. The presence of the epitope tag on the NH2 or COOH terminus did not effect the overall surface expression of CD98hc as measured with an anti-CD98hc antibody (Fig. 3A). Furthermore, both the NH2- and COOH-terminal tags were present on the expressed protein (Fig. 3B). Consequently, we conclude that the NH2 terminus of CD98hc is intracellular, and the COOH terminus is extracellular.

**COOH Terminus Is Extracellular**—In the foregoing experiments, we found that the interactions of CD98 with integrins could be ascribed to its heavy chain. Consequently, we wished to analyze the structural determinants in the heavy chain responsible for these interactions. CD98hc is predicted to be a type II transmembrane protein, with the COOH terminus extracellular and the NH2 terminus intracellular. To document the membrane topography of CD98hc, the NH2 and COOH terminus of CD98hc were separately tagged using a HA-tag. When cells were transfected with the COOH-terminal-tagged cDNA, the epitope tag was readily detected on the cell surface as indicated by a rightward shift in the fluorescence intensity (Fig. 3A). In contrast, the amino-terminal tag was not detected on the cell surface. The presence of the epitope tag on the NH2 or COOH terminus did not effect the overall surface expression of CD98hc as measured with an anti-CD98hc antibody (Fig. 3A). Furthermore, both the NH2- and COOH-terminal tags were present on the expressed protein (Fig. 3B). Consequently, we conclude that the NH2 terminus of CD98hc is intracellular, and the COOH terminus is extracellular.

**The Cytoplasmic and Transmembrane Domains of CD98hc Are Required for Its Interaction with Integrins**—Having confirmed the membrane topography of CD98hc, we wished to examine the role of its cytoplasmic and extracellular domains in the integrin interaction. We first deleted the cytoplasmic domain of CD98hc (Fig. 4A). This abolished its ability to complement dominant suppression (Fig. 4B), although it did not block surface expression (data not shown, but see Fig. 5). Deletion of the CD98hc cytoplasmic domain completely abolished its ability to bind to the β1A cytoplasmic domain (Fig. 4A). This abolished its ability to complement dominant suppression (Fig. 4B), although it did not block surface expression (data not shown, but see Fig. 5). Deletion of the CD98hc cytoplasmic domain completely abolished its ability to bind to the β1A cytoplasmic domain (Fig. 4A). As an alternative approach, we exchanged the cytoplasmic domain of CD98hc with another type II transmembrane protein (CD69). That chimera (C<sub>α</sub>T<sub>α</sub>E<sub>β</sub>) failed to complement dominant suppression (Fig. 4B) and failed to bind to the β1A cytoplasmic...
The cytoplasmic domain of CD98hc is required for integrin interactions. A, model of CD98hc/CD69 chimeras. B, complementation of dominant suppression (CDS): αβγ cells were transfected with Tac-β1 and the CD98hc constructs depicted in A. Twenty-four h after transfection, cells were collected and the Tac-positive subset of cells were analyzed for the ability to bind to the PAC1 antibody. Data are expressed as percentage reversal of Tac-β1A suppression, as described in the legend to Fig. 1. C, binding to β1A tail: affinity chromatography with β1A or αβγ tails was performed using lysates of surface-biotinylated CHO cells that had been transfected with the constructs described in A. Bound proteins that contain the extracellular domain of CD98hc (panels a, b, and c) were eluted from the beads, immunoprecipitated with anti-CD98hc antibody, fractionated by SDS-PAGE, and the biotinylated polypeptides were detected by streptavidin-peroxidase chemiluminescence. Bound proteins that contain the extracellular domain of CD69 (panel d) were eluted from the beads, immunoprecipitated with anti-CD69 antibody.

Fig. 4. The cytoplasmic domain of CD98hc is required for integrin interactions. A, model of CD98hc/CD69 chimeras. B, complementation of dominant suppression (CDS): αβγ cells were transfected with Tac-β1 and the CD98hc constructs depicted in A. Twenty-four h after transfection, cells were collected and the Tac-positive subset of cells were analyzed for the ability to bind to the PAC1 antibody. Data are expressed as percentage reversal of Tac-β1A suppression, as described in the legend to Fig. 1. C, binding to β1A tail: affinity chromatography with β1A or αβγ tails was performed using lysates of surface-biotinylated CHO cells that had been transfected with the constructs described in A. Bound proteins that contain the extracellular domain of CD98hc (panels a, b, and c) were eluted from the beads, immunoprecipitated with anti-CD98hc antibody, fractionated by SDS-PAGE, and the biotinylated polypeptides were detected by streptavidin-peroxidase chemiluminescence. Bound proteins that contain the extracellular domain of CD69 (panel d) were eluted from the beads, immunoprecipitated with anti-CD69 antibody.

The cytoplasmic and transmembrane domains of CD98hc are necessary and sufficient for binding to β1A cytoplasmic domains. CHO cells were transfected with each of the chimeric cDNAs depicted on the left-hand side of the figure. Twenty-four h later, surface proteins were labeled with Sulfo-Biotin N-hydroxysuccinimide, and the cells were lysed. Cell lysates were incubated with beads coated with model proteins containing β1A or αβγ cytoplasmic tails. Bound and eluted proteins that contain the extracellular domain of CD98hc (CD98hc, C98T69E98) were immunoprecipitated with anti-CD98hc antibody and fractionated by reduced SDS-PAGE, and the biotinylated polypeptides were detected by streptavidin-peroxidase chemiluminescence. Bound proteins that contain the extracellular domain of CD69 (CD69, C98T69E98, C98T69E69) were detected by immunoblot with the anti-CD69 antibody.

The cytoplasmic and transmembrane domains of CD98hc are necessary and sufficient for binding to β1A cytoplasmic domains. CHO cells were transfected with each of the chimeric cDNAs depicted on the left-hand side of the figure. Twenty-four h later, surface proteins were labeled with Sulfo-Biotin N-hydroxysuccinimide, and the cells were lysed. Cell lysates were incubated with beads coated with model proteins containing β1A or αβγ cytoplasmic tails. Bound and eluted proteins that contain the extracellular domain of CD98hc (CD98hc, C98T69E98) were immunoprecipitated with anti-CD98hc antibody and fractionated by reduced SDS-PAGE, and the biotinylated polypeptides were detected by streptavidin-peroxidase chemiluminescence. Bound proteins that contain the extracellular domain of CD69 (CD69, C98T69E98, C98T69E69) were detected by immunoblot with the anti-CD69 antibody.

of CD98hc were required for its capacity to bind to the β1A cytoplasmic domain. To investigate whether the functional effects of CD98hc correlated with its binding to the β1A cytoplasmic domain, each of these chimeras (Fig. 6A) was analyzed for its capacity to mediate CDS and to promote Ile transport. In these experiments, the expression of each chimera was verified by flow cytometry, and equivalent expression was observed for each one (data not shown). As previously noted, the substitution of the cytoplasmic domain of CD98hc with that of CD69 abolished CDS (C98T69E98; Fig. 6B). However, this chimera stimulated Ile transport to comparable levels with wild-type CD98hc (Fig. 6C). Similarly, substitution of the transmembrane domain of CD98hc (C69T69E98) markedly suppressed effects on integrin function (Fig. 6B) but had little effect on the ability to stimulate Ile transport (Fig. 6C). Thus, the amino acid transport activity of CD98hc is not sufficient for CDS. Conversely, the substitution of the extracellular domain of CD98hc with that of CD69 (C98T69E69) preserved effects on integrin function (Fig. 6B) but abolished amino acid transport activity (Fig. 6C). Thus, the extracellular domain of CD98hc is necessary (in the context of another type II transmembrane protein) for its ability to stimulate isoleucine transport. Conversely, the transmembrane and cytoplasmic domains of CD98hc are necessary and sufficient for binding to the β1A tail and for augmentation of integrin function.

**DISCUSSION**

CD98hc combines with several different light chains to form a series of heterodimers that are involved in amino acid transport. CD98hc binds to integrin β1A cytoplasmic domains and blocks the capacity of β1A cytoplasmic domains to suppress integrin activation (CDS). We have compared the structural requirements of CD98hc for interaction with integrins with those involved in regulation of amino acid transport. Here we report that: 1) mutation of cysteines that disrupt CD98 heavy-light chain association and reduce amino acid transport do not disrupt its binding to β1A or its effect on integrin activation. 2) The cytoplasmic and transmembrane domains of CD98hc fused to another type II transmembrane protein are both necessary and sufficient for binding to the integrin β1A tail and for CDS. This chimera failed to stimulate amino acid transport.
The cytoplasmic and transmembrane domains of CD98hc are necessary and sufficient for its effect on integrin function, but not amino acid transport. A, model of CD98hc/CD69 chimeras. B, COS-7 αβ2Py cells were transfected with Tac-β1 and the CD98hc chimeras depicted in A. Twenty-four h after transfection, cells were collected, and the Tac-positive subset of cells were analyzed for the ability to bind to the PAC1 antibody. Data are expressed as percentage reversal of Tac-β1 suppression, as described in the legend to Fig. 1. C, amino acid transport: αβ2Py cells were transfected with cDNAs encoding the hLAT1 light chain and the CD98hc chimeras depicted in A. The uptake of [3H]isoleucine was measured 24 h after transfection as described under “Experimental Procedures.” The uptake was measured in a Na+-free solution, and the values are expressed as cpm/mg protein, where the base-line uptake in cells transfected with hLAT1 alone has been subtracted.

3) Replacement of the cytoplasmic or transmembrane domains of CD98hc with those of CD69 blocked the capacity of CD98hc to bind to β1A and regulate integrin activation, but had minimal effects on the amino acid transport function of CD98. Thus, the amino acid transport function of CD98 is not required for its effects on integrin function, and amino acid transport can occur in the absence of CD98hc-integrin association.

The formation of a covalent CD98 heterodimer is not required for its effects on integrin function. CD98hc has two extracellular cysteines Cys109 and Cys330. Cys109 is near the transmembrane domain of CD98hc and results in a disulfide bridge with a cysteine in an extracellular loop of the light chain between transmembrane domains 3 and 4 (25). Mutation of Cys109 and Cys330 disrupted the covalent association with the light chain but did not impair interactions with or effects on integrins. While the covalent association was lost, it is possible that there was still a noncovalent interaction. Indeed, Pfeifer et al. (25) reported that the C109S mutant does still support the surface expression of the light chain. The C109S mutation still displays the same transport characteristics as the disulfide-bound heterodimers, albeit at a reduced rate. Moreover, we also found that overexpressed free heavy chains could also bind to the β1A tail. Furthermore, cotransfection of the hLAT1 light chain increased formation of heterodimers and amino acid transport but did not augment integrin interactions or effects. Consequently, our results indicate that the covalent association of CD98hc with a light chain is not required for its interaction with integrins or for the functional regulation of integrins.

The cytoplasmic and transmembrane domains of CD98hc are both necessary and sufficient for binding to the integrin β1A tail and for effects on integrin function. When either of these domains was removed from CD98hc, integrin interactions were lost. Conversely, effects on integrins could be conveyed to CD69 by addition of these two domains. What is the role of the CD98hc transmembrane domain in binding to the β1A cytoplasmic tail? It is possible that the CD98hc transmembrane domain influences the conformation of the cytoplasmic domain to promote binding to integrin cytoplasmic domains. Alternatively, our integrin cytoplasmic domain model protein was based on that predicted from the sequence (ITB1_human) in the Swiss-Prot data base (Entry P05556). Glycosylation mapping studies have suggested that β1A (Lys752–Ile757) of the predicted “cytoplasmic” domain may reside in the membrane (27). Consequently, the CD98hc transmembrane domain may directly interact with a transmembrane portion of our model protein “tail.” Furthermore, other integrin-binding proteins, such as cyttoesin-1, Rack1, and skelemain also bind the membrane proximal region (28). Thus, the localization of this region in the membrane may specify preferential binding of integrin-associated proteins. Finally, the CD98hc solubilized from membranes could be associated with other proteins via the transmembrane domains. These “adapters” might contribute to the CD98hc-β1A tail interaction. In any case, our studies provide the first delineation of a specific functional role for the cytoplasmic and transmembrane domains of CD98hc, interaction with and regulation of β1A integrin function.

Regulation of amino acid transport and integrins by CD98hc is a distinct and separable function of the polypeptide. Chimeras in which the cytoplasmic or transmembrane domains of CD98hc were replaced with those of CD69 lost the capacity to bind to β1A and regulate integrin activation. In contrast, these replacements had little effect on the amino acid transport function of CD98. Conversely, the exchange of the extracellular domain of CD98hc with that of CD69 resulted in a protein that was still capable of affecting integrin function but did not stimulate isoleucine transport. Thus, the amino acid transport activity of CD98hc is not required for its effect on integrin function.

CD98hc functions as a chaperone to bring the associated light chains (LAT1, LAT2, y1LAT1, y1LAT2, xCT, and asc-1) to the plasma membrane (29, 30). We found that the interaction of CD98hc with integrins and amino acid transporters are ascribable to distinct domains of the protein and are not mutually exclusive. Integrin-mediated adhesion often leads to the polarization of these receptors to the adherent cell surface. Consequently, the integrin-CD98hc interaction may serve to polarize the localization of CD98 and, consequently, amino acid transport. Conversely, CD98hc can influence multiple integrin-dependent functions, including virus-induced cell fusion, T-cell costimulation, and cell adhesion (13, 15–17). Thus, the CD98hc-integrin association can promote integrin-mediated cell adhesion that, in turn, could serve to localize the activities of CD98hc-linked amino acid transporters.

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REFERENCES

1. Haynes, B. F., Hemler, M. E., Mann, D. L., Eisenbarth, G. S., Shelhamer, J., Mostowski, H. S., Thomas, C. A., Strominger, J. L., and Fauci, A. S. (1981) J. Immunol. 126, 1409–1414
2. Yagita, H., Masuko, T., and Hashimoto, Y. (1986) Cancer Res. 46, 1478–1484
3. Warren, A. P., Patel, K., McConkey, D. J., and Palacios, R. (1996) Blood 87, 3676–3687
4. Verrey, F., Meier, C., Rossier, G., and Kuhn, L. C. (2000) Pfluegers Arch. 440, 503–512
5. Calonge, M. J., Gasparini, P., Chillaron, J., Chillon, M., Gallucci, M., Rousaud, F., Zelante, L., Testar, X., Dallapiccola, B., and Di Silverio, F. (1994) Nat. Genet. 6, 420–425
6. Mastroberardino, L., Spindler, B., Pfeiffer, R., Skelly, P. J., Loffing, J., Shoemaker, C. B., and Verrey, F. (1998) Nature 395, 288–291
7. Torrens, D., Estevez, R. A., Pineda, M., Fernandez, E., Lloberas, J., Yun-Ba, S., Zerzato, A., and Palacin, M. (1998) J. Biol. Chem. 273, 32437–32445
8. Kanai, Y., Segawa, H., Miyamoto, K., Chimino, H., Takeda, E., and Endou, H. (1998) J. Biol. Chem. 273, 36769–36772
9. Nakamura, F., Sato, M., Yang, H., Miyagawa, F., Harasaki, M., Tomita, K., Matsumoto, S., Nonaka, K., Iwai, K., and Minato, N. (1999) J. Biol. Chem. 274, 3009–3016
10. Hynes, R. O. (1992) Cell 69, 11–25
11. Hughes, P. E., and Pfaff, M. (1998) Trends Cell Biol. 8, 359–364
12. Chen, Y.-P., O'Toole, T. E., Shipley, T., Forsyth, J., LaFlamme, S. E., Yamada, K. M., Shattil, S. J., and Ginsberg, M. H. (1994) J. Biol. Chem. 269, 18307–18310
13. Fenczik, C. A., Sethi, T., Ramos, J. W., Hughes, P. E., and Ginsberg, M. H. (1997) Nature 390, 81–85
14. Zent, R., Fenczik, C. A., Calderwood, D. A., Liu, S., Dellos, M., and Ginsberg, M. H. (2000) J. Biol. Chem. 275, 5505–5506
15. Ohta, H., Tsurudome, M., Matsumura, H., Koja, Y., Morikawa, K., Kawanoue, M., Kusugawa, S., Komada, H., Nishio, M., and Ito, Y. (1994) EMBO J. 13, 2044–2055
16. Chandrasekaran, S., Guo, N. H., Rodrigues, R. G., Kaiser, J., and Roberts, D. D. (1999) J. Biol. Chem. 274, 11408–11416
17. Warren, A. P., Patel, K., Miyamoto, Y., Wygant, J. N., Woodside, D. G., and McIntyre, B. W. (2000) Immunology 99, 62–68
18. Shattil, S. J., Hoxie, J. A., Cunningham, M., and Brass, L. F. (1985) J. Biol. Chem. 260, 11107–11114
19. Frelinger, A. L., III, Du, X., Plow, E. F., and Ginsberg, M. H. (1991) J. Biol. Chem. 266, 17106–17111
20. Ali, L., Edelhauser, A., Hadzir, P., Hurzeler, M., Knopp, D., Muller, M., Steiner, B., Trzezak, A., and Weller, T. (1992) J. Med. Chem. 35, 4393–4407
21. Pfaff, M., Liu, S., Erle, D. J., and Ginsberg, M. H. (1998) J. Biol. Chem. 273, 6104–6109
22. LaFlamme, S. E., Thomas, L. A., Yamada, S. S., and Yamada, K. M. (1994) J. Cell Biol. 126, 1287–1298
23. Baker, E. K., Tozer, E. C., Pfaff, M., Shattil, S. J., Lofts, J. C., and Ginsberg, M. H. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1973–1978
24. O'Toole, T. E., Katagiri, Y., Faull, R. J., Peter, K., Tamura, H. N., Quaranta, V., Lofts, J. C., Shattil, S. J., and Ginsberg, M. H. (1994) J. Cell Biol. 124, 1047–1059
25. Pfaff, R., Spindler, B., Loffing, J., Shoemaker, C. B., and Verrey, F. (1998) FEBS Lett. 439, 157–162
26. Estevez, R., Campo, M., Rojas, A. M., Testar, X., Deves, R., Hediger, M. A., Zorzano, A., and Palacin, M. (1998) FASEB J. 12, 1319–1329
27. Armulik, A., Nilsson, I., von Heijne, G., and Johansson, S. (1999) J. Biol. Chem. 274, 37030–37034
28. Liu, S., Calderwood, D. A., and Ginsberg, M. H. (2000) J. Cell Biol. 113, 3563–3571
29. Verrey, F., Jack, D. L., Paulsen, I. T., Saier, M. H., Jr., and Pfaff, R. (1999) J. Biol. Chem. 117, 181–192
30. Palacin, M., Bertran, J., and Zorzano, A. (2000) Curr. Opin. Nephrol. Hypertens. 9, 547–553