CD98 is a type II transmembrane protein involved in neutral and basic amino acid transport and in cell fusion events. CD98 was implicated in the function of integrin adhesion receptors by its capacity to reverse suppression of integrin activation by isolated integrin β1A domains. Here we report that CD98 interacts with integrin β cytoplasmic domains with a unique integrin class and splice variant specificity. In particular, CD98 interacted with the ubiquitous β1A but not the muscle-specific splice variant, β1D, or leukocyte-specific β2 cytoplasmic domains. The ability of CD98 to associate with integrin cytoplasmic domains correlated with its capacity to reverse suppression of integrin activation. The association of CD98 with integrin β1A cytoplasmic domains may regulate the function and localization of these membrane proteins.

The development and function of multicellular animals requires integrin adhesion receptors (1). Integrin-dependent cell adhesion is regulated, in part, by ligand binding affinity (“activation”) changes controlled by cellular signaling cascades (1–3). Regulation of integrin affinity is important in cell migration (4–6), extracellular matrix assembly (7), and morphogenesis (8). Integrin activation is energy-dependent and is mediated by cell type specific signals operating through integrin cytoplasmic domains (9).

Complementation of dominant suppression (CODS) is an expression cloning scheme used to identify proteins that modulate integrin affinity (10). CODS depends on the ability of an isolated integrin β1A cytoplasmic domain, in the form of a chimera with the α subunit of the interleukin-2 receptor, to block integrin activation (dominant suppression). Proteins involved in integrin activation are isolated by their ability to complement dominant suppression. CD98, a type II transmembrane protein first discovered as a T-cell activation antigen (11), was identified utilizing CODS. CD98, although widely expressed on proliferating cells, is generally down-regulated in quiescent cells (12). CD98 forms disulfide-bonded heterodimers with several light chains that strongly resemble permeases (13–20). CD98 regulates the transport of neutral and positively charge amino acids through these light chains (14, 15, 17, 18). Thus, CODS has identified an unexpected connection between cell adhesion and certain amino acid transporters.

The mechanism by which CD98 influences integrin function is not yet clear. CODS was predicated on the idea that it would identify integrin β cytoplasmic domain binding proteins (10). Many β cytoplasmic domains manifest overall sequence similarity (1, 2); however, the cytoskeletal protein, talin, binds to the muscle-specific splice variant, β1D, more tightly than to β1A. In addition, the leukocyte-specific β2 cytoplasmic domain binds to filamin more tightly than to β1A (21). We have now examined interactions between CD98 and recombinant parallel-dimerized integrin β1A, β1D, and β2 cytoplasmic domains by affinity chromatography (21). Here we report that CD98 interacts with the β1A but not β1D or β2 integrin cytoplasmic domains. Furthermore, the CD98 interaction is insensitive to β cytoplasmic domain mutations that abolish the binding of talin and filamin. The capacity of CD98 to complement dominant suppression correlates with its capacity to bind to the suppressive β cytoplasmic domains. The interaction of the integrin β1A cytoplasmic domain with CD98 may thus serve to regulate the localization and the function of these membrane proteins.
CD98 Binds Integrin β Tails

FIG. 1. Amino acid sequences of integrin β cytoplasmic domains. Depicted is an alignment of the integrin cytoplasmic domains used in this study. The underlined tyrosine (Y) was mutated to an alanine (A) to form the YA mutants. All integrin sequences with the exception of β1 correspond to those human sequences published in the Swiss-Protein Data base as of May 15, 1999. In β2, the amino-terminal Arg was changed to Lys in order to introduce a 2H2O. H2O with protease inhibitors) was added to the 2H2O. H2O, 16 mM MgSO4, pH 7.3–7.4), suspended in a 1:1 mixture of 20 mM Hepes and buffer B. The membrane fraction was resuspended in buffer A, 1 mM EDTA, and protease inhibitors and an equal quantity of buffer B (20 mM Hepes, 0.5 M sucrose, 10 mM MgCl2, 0.1 M NaCl, 20 mM Tris-HCl, pH 7.9) and 1 ml of immunoprecipitation buffer containing 1% Triton X-100, 0.5% sodium deoxycholate, 1 mM EDTA, and protease inhibitors (aprotinin, 5 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride). Platelet lysates were prepared as described previously (21).

Subcellular fractionation of Jurkat cells was performed after surface biotinylation. The cells were washed three times in Hepes-saline (200 mM Hepes, 12 mM CaCl2, 2H2O, 16 mM MgSO4, pH 7.3–7.4), suspended in 20 mM Hepes, and homogenized with a Dounce homogenizer. An equal quantity of buffer B (20 mM Hepes, 0.5 M sucrose, 10 mM MgCl2, 0.1 M NaCl, 2 mM CaCl2-H2O with protease inhibitors) was added to the homogenate, and the mixture was centrifuged at 500 × g for 30 min at a Beckman model L7-65 centrifuge. The cytoplasmic fraction (supernatant) was removed and the membrane fraction (pellet) washed in a 1:1 mixture of 20 mM Hepes and buffer B. The membrane fraction was resuspended in buffer A, 1 mM EDTA, and protease inhibitors and centrifuged at 30,000 × g for 20 min.

Affinity Chromatography Experiments—Recombinant proteins were expressed in BL21(DE3)pLysS cells (Novagen) and bound to His-bind resin (Novagen) through their N-terminal His tag in a ratio of 1 ml of beads/ liter of culture. Coated beads were washed with PN (20 mM Pipes, 160 mM NaCl, 20 mM Tris-HCl, pH 7.4) and stored at 4°C. In an equal volume of PN containing 0.1% NaN3, beads were added to cell lysates diluted in buffer A. (0.05% Triton X-100, 3 mM MgCl2, and protease inhibitors) and incubated overnight at 4°C and then washed five times with buffer A. 100 μl of SDS-sample buffer was added to the beads and the mixture was heated at 100°C for 5 min. After 10,000 rpm centrifugation in a microcentrifuge, the supernatant was fractionated by SDS-PAGE and analyzed by Western blotting. In some experiments, the beads were eluted from the beads with 100 μl of elution buffer (1X imidazole, 500 mM NaCl, 20 mM Tris-HCl, pH 7.9) and 1 ml of immunoprecipitation buffer containing 1% Triton X-100, 0.05% Tween 20, and protease inhibitors) was then added. The eluted proteins were immunoprecipitated overnight at 4°C with an 4F2 antibody pre-bound to protein A-Sepharose beads (Amersham Pharmacia Biotech). The following day, the beads were washed three times with the immunoprecipitation buffer and heated in reduced sample buffer for SDS-PAGE under reducing conditions. Samples were separated on 4–20% SDS-polyacrylamide gels (Novex) and transferred to nitrocellulose membranes. Membranes were blocked with Tris-buffered saline, 5% nonfat milk powder and 0.1% stained with streptavidin-peroxidase or with specific antibodies and appropriate peroxidase conjugates. Bound peroxidase was detected with an enhanced chemiluminescence kit (Amersham Pharmacia Biotech). Equal loading of NaI32+ beads with recombinant proteins were verified by Coomassie Blue staining of SDS-PAGE profiles of SDS eluted proteins.
lated Jurkat cell lysates were allowed to bind to model proteins containing a heterodimer of the βIIb (βIIb) or αIIb (αIIb) cytoplasmic tails. Adjacent lanes show the surface proteins present in the lysate (lysat) or the ones that bound to uncoated Ni²⁺ beads (0). In panel B, the biotinylated surface proteins that bound to the βIIb (βIIb) or αIIb (αIIb) tails or uncoated beads (0) were immunoprecipitated with CD98 antibody (IP) or a control IgG (IgG). The immunoprecipitates were fractionated by reduced SDS-PAGE, and biotinylated proteins were detected by streptavidin-peroxidase-generated chemiluminescence.

Fig. 2. β1A cytoplasmic domains bind CD98. Jurkat human T cells were surface-labeled with Sulfo-Biotin N-hydroxysuccinimide, and the cells were lysed in buffer A (see “Experimental Procedures”). Panel A depicts a reduced SDS-PAGE analysis of the biotinylated proteins that bound to Ni²⁺ beads, coated with model proteins containing β1A (β1A) or αIIb (αIIb) cytoplasmic tails. Adjacent lanes show the surface proteins present in the lysate (lysat) or the ones that bound to uncoated Ni²⁺ beads (0). In panel B, the biotinylated surface proteins that bound to the β1A (β1A) or αIIb (αIIb) tails or uncoated beads (0) were immunoprecipitated with CD98 antibody (IP) or a control IgG (IgG). The immunoprecipitates were fractionated by reduced SDS-PAGE, and biotinylated proteins were detected by streptavidin-peroxidase-generated chemiluminescence.

binding to β1D, nor is it required for CD98 binding to β1A.

The Y788A mutation of β1A (Fig. 1) disrupts filamin (Fig. 4B) and talin (Fig. 5C) binding (21). Similar Tyr to Ala mutations in β7 and β1D tails, corresponding to the Y788A mutation in β1A (Fig. 1), also disrupted filamin (Fig. 4B) and talin (Fig. 5C) binding. CD98 binding to β integrin tails was not affected by Tyr to Ala mutations (Figs. 4B and 5C). The Tyr to Ala mutation introduced into β1D or β7 did not increase CD98 binding, nor was CD98 binding reduced in the β1A(Y788A) mutant. These results confirm that talin or filamin competition does not account for the lack of CD98 binding to β1D and β7 and that talin or filamin binding is not required for CD98 binding to the β1A cytoplasmic domain.

CD98 Binding to Integrin Cytoplasmic Domains Correlates
CD98 Binds Integrin β Tails

CD98 Binding Is Not Sufficient to Induce Dominant Suppression—As noted above, β1A tails suppress integrin activation and bind CD98. To assess whether CD98 binding alone is sufficient to induce dominant suppression, we first examined CD98 binding to a series of β1A truncation mutants (Fig. 1). CD98 binding was lost when the C-terminal seven residues were deleted (β1AC797X) but not when the last three amino acids were eliminated (β1A(801X)) (Fig. 7A). Despite maintaining its capacity to bind to CD98, the Tac-β1A(801X) mutant was a poor suppressor of integrin activation (Fig. 7B), and this was not due to a quantitative reduction in the association of CD98 with β1A(801X) (Fig. 7C). Furthermore, the β1A(788A) mutant, which also bound CD98 (Figs. 4 and 5), failed to suppress integrin activation (Fig. 7B). Consequently, integrin β cytoplasmic domain binding to CD98 is not sufficient to induce dominant suppression.

DISCUSSION

CD98 is implicated in several cellular functions, including amino acid transport, cell fusion events, and integrin activation (12). We previously found that CD98 reverses dominant suppression of integrin function (10). We now report that: 1) CD98 associates with the β1A integrin cytoplasmic domain; 2) CD98 interacts differentially with β cytoplasmic tails in a class- and splice variant-specific manner, which is independent of the capacity of the tails to bind the cytoskeletal proteins talin and filamin; 3) CD98’s capacity to associate with integrin tails correlates with its ability to overcome dominant suppression of integrin activation; 4) CD98 association with integrin tails is neither necessary nor sufficient for dominant suppression of integrin activation. Thus, the association of CD98 with integrin cytoplasmic domains may regulate the function and localization of these membrane proteins.

CD98 physically associates with β1A integrin cytoplasmic domains. This association was observed utilizing model protein mimics of dimerized integrin cytoplasmic tails, and it may account for the physical association of certain β1 integrins with CD98.2 The specificity of the interaction was confirmed by the lack of binding to mimics containing cytoplasmic domains from αIIb or several other β subunits. CD98 was added to the tails in the presence of other cellular proteins, so it remains possible that an intermediary protein is required for this interaction. However, CD98 was the only surface protein observed binding to the β1A tail (Fig. 2). Moreover, we observed CD98 binding in the absence of two known integrin binding proteins, talin and filamin (Figs. 3 and 4). CD98 failed to bind to β1D and β7 cytoplasmic domains, even though these tails bind many of the same polypeptides as β1A (21). Thus, we conclude that CD98 associates with the β1A tail and that the interaction is potentially direct.

CD98 binds to integrin β cytoplasmic domains with unique splice variant and class specificity. CD98 bound well to the β1A tail and the β3 tail. Binding to the β1D and β7 tails was negligible. The specificity of CD98 binding differs markedly from the specificity of talin and filamin binding, since talin binds preferentially to the β1D tail and filamin to the β7 tail (21). Moreover, the binding of both cytoskeletal proteins is sensitive to the Tyr substitution with Ala in the first “NPXY” (21) in β1A and, as shown here, in β3 and β7. Strikingly, CD98 binding was insensitive to this mutation. Finally, although the last three residues of β1A were dispensable, the last seven residues were required for binding. Thus, the features of the β tail defined here for CD98 binding identifies a novel structural specificity for integrin β tail function.

CD98 binding to β tails correlates with its capacity to com-

with Complementation of Dominant Suppression—Overexpression of isolated integrin β1A cytoplasmic domains, in the form of a Tac-β1A chimera, results in suppression of integrin activation. Dominant suppression is reversed by overexpression of CD98 (10). Tac-β1A, Tac-β1D, and Tac-β7 induced dominant suppression of integrin activation (Fig. 6A). As noted above (Fig. 3), CD98 bound poorly to β1D and β7 tails, showing that CD98 binding is not required for dominant suppression. However, CD98 was much less effective at reversing the suppression induced by Tac-β1A and Tac-β7 (Fig. 6B). Thus the capacity of CD98 to rescue suppression correlates with its binding to the suppressive β cytoplasmic domain.

2 M. Hemler, personal communication.
plement dominant suppression. CD98 was implicated in integrin activation by its capacity to reverse the suppression of integrin activation caused by an isolated β1A cytoplasmic domain (10). In the present work, we found that CD98 binds to the β1A cytoplasmic domain, but fails to bind well to the β2 or β1D cytoplasmic domain. Strikingly, CD98 failed to complement dominant suppression initiated by either β2 or β1D cytoplasmic domains. Consequently, the mechanism of CDS appears to involve CD98 binding to the suppressive β tail. Furthermore, cross-linking of CD98 stimulates integrin αβ-dependent adhesion in small cell lung cancer cells (10) and in certain breast cancer cell lines (28) and β1 integrin-dependent cell fusion events (29–36). Thus, our finding that CD98-β1 cytoplasmic domain interactions correlate with effects on integrin function is relevant to integrin-dependent events involved in multilamellar giant cell formation, virally induced cell fusion, and regulation of cell adhesion.

The physical interaction of CD98 with integrin cytoplasmic domains may be involved in modulating amino acid transport regulation. CD98 is known to regulate γ+L and L type amino acid transport (14, 15, 17, 18). This regulation is probably due to disulfide-bonded heterodimer formation with a variety of light chains, that resemble permease amino acid transporters (13–20). In fact, mutations in one of these light chains (15) are a likely cause of lysinuric protein intolerance (37). CD98 may function to regulate both the expression and localization of its light chains (18). In certain cells CD98 has a basolateral localization (38). β1A integrins also manifest basolateral polarization in many cells (39, 40), probably due to interactions with underlying matrix components (41) or recruitment to lateral cell contacts (42). It is noteworthy that β1 integrins are primarily involved in lymphocyte homing and β1 integrins primarily form mechanical linkages in striated and cardiac muscle (43, 44). Thus, the failure of these cytoplasmic domains to bind to CD98 correlates well with their lack of a role in establishing polarity in epithelial or mesenchymal cells. Consequently, the physical association of CD98 with β1A integrin cytoplasmic domains may participate in the polarization and regulation of amino acid transporters and to modulate the function of certain integrins.

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REFERENCES
1. Hynes, R. O. (1992) Cell 69, 11–25
2. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Dev. Biol. 11, 549–599
3. Hughes, P. E., Renshaw, M. W., Pfaff, M., Forysth, J., Keivens, V. M., Schwartz, M. A., and Ginsberg, M. H. (1997) Cell 88, 521–530
4. Hutenlocher, A., Ginsberg, M. H., and Horwitz, A. F. (1996) J. Cell Biol. 134, 1551–1562
5. Hutenlocher, A., Palecek, S. P., Lu, Q., Zhang, W., Mellgren, R. L., Lauffenburger, D. A., Ginsberg, M. H., and Horwitz, A. F. (1997) J. Biol. Chem. 272, 32719–32722
6. Filardo, E. J., Brookes, P. C., Deming, S. L., Damsky, C., and Cheresh, D. A. (1993) J. Cell Biol. 120, 441–450
7. Wu, C., Keivens, V. M., O’Toole, T. E., McDonald, J. A., and Ginsberg, M. H. (1995) Cell 83, 715–724
8. Martin-Bernadou, M. D., Danin-Borkowski, O. M., and Brown, N. H. (1998) J. Cell Biol. 141, 1573–1581
9. O’Toole, T. E., Katagiri, Y., Faull, R. J., Peter, K., Tamura, R. N., Quaranta, V., Loftus, J. C., Shattil, S. J., and Ginsberg, M. H. (1994) J. Cell Biol. 124, 1047–1059
10. Fenech, C. A., Sethi, T., Ramos, J. W., Hughes, P. E., and Ginsberg, M. H. (1997) Nature 387, 81–85
11. 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
12. Diaz, L. A., Jr., and Fox, D. A. (1998) J. Biol. Chem. 273, 32719–32722
13. Mannion, B. A., Kolesnikova, T. V., Lin, S. W., Wang, S., Thompson, N. L., and Hemler, M. E. (1998) J. Biol. Chem. 273, 32719–32722
15. Torrents, D., Estevez, R., Pineda, M., Fernandez, E., Lloberas, J., Shi, Y.-B., Zorzano, A., and Palacin, M. (1998) *J. Biol. Chem.* 273, 32437–32445
16. Estevez, R., Camps, M., Rojas, A. M., Teszar, X., Deves, R., Hediger, M. A., Zorzano, A., and Palacin, M. (1998) *FASEB J.* 12, 1319–1329
17. Mastroberardino, L., Spindler, B., Pfeiffer, R., Loffing, J., Skelley, P. J., Shoemaker, C. B., and Verrey, F. (1998) *Nature* 395, 288–291
18. Pfeiffer, R., Rossier, G., Spindler, B., Meier, C., Kuhn, L., and Verrey, F. (1999) *EMBO J.* 18, 49–57
19. Pfeiffer, R., Spindler, B., Loffing, J., Skelley, P. J., Shoemaker, C. B., and Verrey, F. (1998) *FEBS Lett.* 439, 157–162
20. Tsurudome, M., Ito, M., Takebayashi, S., Okumura, K., Nishio, M., Kawano, M., Komada, H., Sakagawa, S., Kusawaga, S., Komada, S., and Ito, Y. (1999) *J. Immunol.* 162, 2462–2466
21. Pfaff, M., Liu, S., Erle, D. J., and Ginsberg, M. H. (1998) *J. Biol. Chem.* 273, 6104–6109
22. Shattil, S. J., Hoxie, J. A., Cunningham, M., and Brass, L. F. (1985) *J. Biol. Chem.* 260, 11107–11114
23. Frelinger, A. L., III, Du, X., Plow, E. F., and Ginsberg, M. H. (1991) *J. Biol. Chem.* 266, 17106–17111
24. Alig, L., Edenhofer, A., Hadvary, P., Huzaeler, M., Knopp, D., Muller, M., Steiner, B., Trzeciak, A., and Weller, T. (1992) *J. Med. Chem.* 35, 4393–4407
25. LaFlamme, S. E., Thomas, L. A., Yamada, S. S., and Yamada, K. M. (1994) *J. Cell Biol.* 126, 1287–1298
26. Baker, E. K., Tozer, E. C., Pfaff, M., Shattil, S. J., Loftus, J. C., and Ginsberg, M. H. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 1973–1978
27. Cunningham, C. C., Gorlin, J. B., Kwiatkowski, D. J., Hartwig, J. H., Janmey, P. A., Byers, H. R., and Stossel, T. P. (1992) *Science* 255, 325–327
28. Chandrasekaran, C., Guo, N., Rodrigues, R. G., Kaiser, J., and Roberts, D. D. (1999) *J. Biol. Chem.* 274, 11408–11416
29. Higuchi, S., Tabata, N., Tajima, M., Ito, M., Tsurudome, M., Okamoto, K., Komada, H., Watanabe, N., and Ito, Y. (1998) *J. Gen. Virol.* 79, 157–162
30. Ohgimoto, S., Tabata, N., Suga, S., Tsururome, M., Kawano, M., Nishio, M., Okamoto, K., Komada, H., Watanabe, N., and Ito, Y. (1996) *J. Immunol.* 157, 3585–3592
31. Ohgimoto, S., Tabata, N., Suga, S., Tsururome, M., Kawano, M., Nishio, M., Okamoto, K., Komada, H., Watanabe, N., and Ito, Y. (1996) *J. Gen. Virol.* 77, 2747–2756
32. Okamoto, K., Ohgimoto, S., Nishio, M., Tsururome, M., Kawano, M., Komada, H., Ito, M., Sakakura, Y., and Ito, Y. (1997) *J. Gen. Virol.* 78, 775–783
33. Okamoto, K., Tsururome, M., Ohgimoto, S., Kawano, M., Nishio, M., Komada, H., Ito, M., Sakakura, Y., and Ito, Y. (1997) *J. Gen. Virol.* 78, 83–89
34. Suga, S., Tsurudome, M., Ito, M., Ohgimoto, S., Tabata, N., Nishio, M., Kawano, M., Komada, H., Sakurakai, M., and Ito, Y. (1997) *Med. Microbiol. Immunol.* 185, 237–243
35. Tabata, N., Ito, M., Shimokata, K., Suga, S., Ohgimoto, S., Tsururome, M., Kawano, M., Matsumura, H., Komada, H., Nishio, M., and Ito, Y. (1994) *J. Immunol.* 153, 3256–3266
36. Ohita, H., Tsururome, M., Matsumura, H., Koga, Y., Morikawa, S., Kawano, M., Kusugawa, S., Komada, H., Nishio, M., and Ito, Y. (1994) *EMBO J.* 13, 2044–2055
37. Torrents, D., Mykkkanen, J., Pineda, M., Feliubadalo, L., Esteves, R. A., de Cic, R., Sanjurjo, P., Zorzano, A., Nunes, V., Huoponen, K., Reinikainen, A., Simell, O., Savontaus, M. L., Aula, P., and Palacin, M. (1999) *Nat. Genet.* 21, 293–296
38. Nakamura, E., Sato, M., Yang, H., Miyagawa, F., Harasaki, M., Tomita, K., Matsuoka, S., Noma, A., Iwai, K., and Minato, M. (1999) *J. Biol. Chem.* 274, 3069–3076
39. Simon, E. E., Liu, C. H., Das, M., Nimag, S., Breckelmann, T. J., and McDonald, J. A. (1994) *Am. J. Physiol.* 267, F612–F623
40. Zambruno, G., Marchisio, P. C., Mareoni, A., Vaschieri, C., Melchiori, A., Giannetti, A., and De Luca, M. (1995) *J. Cell Biol.* 132, 853–865
41. Rahilly, M. A., and Fleming, S. (1995) *J. Pathol.* 176, 297–303
42. Hodivala, K. J., and Watt, F. M. (1994) *J. Cell Biol.* 124, 589–600
43. Belkin, A. M., Zhidkova, N. I., Balzac, F., Altruda, F., Tomatis, D., Maier, A., Tarone, G., Keteliansky, V. E., and Burridge, K. (1996) *J. Cell Biol.* 132, 211–226
44. Belkin, A. M., Retta, S. F., Pletjushkina, O. Y., Balzac, F., Silengo, L., Fassler, R., Keteliansky, V., Burridge, K., and Tarone, G. (1997) *J. Cell Biol.* 138, 1583–1595
Class- and Splice Variant-specific Association of CD98 with Integrin β Cytoplasmic Domains
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