Individual Embryonic Fibroblasts Express Multiple β Chains in Association with the αv Integran Subunit

LOSS OF β3 EXPRESSION WITH CELL CONFLUENCE*

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The α chain of the vitronectin receptor, αv, has been found in association with the integrin subunits β1, β3, or β5 on different cell types. We show here that cultured embryonic fibroblasts simultaneously display αvβ3, αvβ1, and αv in association with two other β subunits, one of which is probably β5. Polymerase chain reaction analysis of single cells isolated by micromanipulation identified mRNA for αv, β1, β3, and β5 in six of eight clones. Immunoprecipitation of iodinated cell surface proteins with a monoclonal antibody to αv indicated that the relative proportions of the different β chains in association with αv varied, particularly between two different cell lines. The cytokines platelet-derived growth factor, transforming growth factor β1, and tumor necrosis factor α did not appear to alter this ratio although tumor necrosis factor α increased the surface expression of the αv-associated integrins; but overnight culture in basic fibroblast growth factor caused a lower expression of αvβ1 and αvβ5 with no reduction in αvβ3 expression. When the cell cultures were grown to complete confluence, surface expression of β3 was abolished, and the expression of an unknown β chain (βa) became more prominent. This effect was not overcome by culturing confluent cells with basic fibroblast growth factor. Affinity column chromatography showed that αvβ5 bound to vitronectin but αvβ1 did not, whereas αvβ1 but not αvβ5 bound to fibronectin. These results suggest that, on individual cells, the β subunits found in association with αv may vary according to the proliferative capacity of the cell and that the promiscuous β3 subunit is progressively replaced by β subunits of individual ligand specificity.

Cell binding to the extracellular matrix provides information signals that greatly influence behavior patterns of migration, proliferation, and differentiation. Among the specific receptors for extracellular matrix proteins is a family of integral cell surface proteins, termed integrins. Each of the integrin receptors comprises an α and β subunit in noncovalent association, and different permutations of α and β chains provide functionally discrete receptors. Currently 11 α chains and seven β chains have been identified, yielding at least 15 known heterodimeric complexes (1), and included in this family are receptors for collagen, fibronectin, vitronectin, laminin, and fibrinogen (reviewed in Refs. 2 and 3).

Initially the integrins were classified into subfamilies based on the specific β subunits (2). It was appreciated that individual β chains could associate with multiple α subunits, and it was considered that the specific properties of the dimeric complexes were provided by the associated α chain. However, one of the subfamilies (that defined by β3) consisted of two members, the platelet glycoprotein GPIIb-IIIa (αIIbβ3) and the vitronectin receptor (αvβ3), and both members are able to bind several matrix proteins (4, 5). More recently, it has also become clear that the α chain of the vitronectin receptor, αv, can associate with several different β subunits in addition to β3 (6–12). Thus, this subunit has been reported to associate with the classical β1 subunit (11, 12) or with a variant of this subunit, termed βn (10). In addition, αv has been found to form a complex with a novel β chain, initially described functionally and biochemically as β6 (7) or βx (6); molecular cloning has shown these molecules to be identical and the novel β chain has been named β5 (13–15). It remains to be determined whether or not another β chain, β3b, associated with αv on macrophages (8) is related to β5 or perhaps to a series of tissue-specific integrin β subunits. Finally, there are data to suggest that an alternatively spliced form of the β3 subunit (16) may also associate with αv on some cells (7).

The complexities introduced by these recent findings have led to the suggestion that the current integrin classification scheme needs revision (11). But they also raise several interesting questions. Specifically, can individual cells simultaneously express more than one β chain in association with αv, and, given that several of the αv-β combinations can bind the same ligand(s) (5–12), what are the conditions that determine which β chain is expressed? Previous studies of cell populations have demonstrated that on different cell types, αv can be found to be associated exclusively with β1 (11, 12), exclusively with β5 (6, 15), or in association with both β1 and β5 (10, 11), although in the latter cases the proportions of β1 to β3 varied considerably on the different cell types and it was not clear whether or not there were different subpopulations of cells. Also, there have been conflicting reports about the ligand specificity of the αv-associated receptor complexes (6, 7, 11, 12), in particular, whether αvβ3 functions as a receptor...
for vitronectin (12) or for fibronectin (11), although such discrepancies may be a reflection of the different cell types used (17).

We have addressed these questions by isolating the mRNA from individual embryonic fibroblast cells and have used the polymerase chain reaction (PCR) to show the simultaneous presence of αv, β1, β3, and β5 in six of the eight clones examined. Immunoprecipitation experiments demonstrated that each of the β chains was in association with αv in cells grown to subconfluence, but that cells grown to complete confluence totally lost αv/β3 expression while retaining surface expression of αvβ1 and αvβ5 and additionally displaying αv in association with another, unidentified β subunit. The presence of both αvβ1 and αvβ5 simultaneously on the same cell also enabled us to compare directly the ligand specificity of these integrins, and it was found that αvβ1 binds fibronectin but not vitronectin, whereas αvβ5 binds vitronectin but not fibronectin.

**EXPERIMENTAL PROCEDURES**

**Materials**—Octyl-β-D-glucopyranoside and Nonidet P-40 were purchased from Boehringer Mannheim, NaCl from Australian Radioisotopes, or from Amersham Corp., the synthetic peptide Gly-Arg-Gly-Asp-Ser-Pro from Auspep Proprietary Ltd., Melbourne, and fibronectin from Collaborative Research Inc. The molecular mass markers used were myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase b (94 kDa), bovine serum albumin (67 kDa), and ovalbumin (45 kDa), and they and other electrophoretic reagents were obtained from Bio-Rad Laboratories. The cytoxin, platelet-derived growth factor was from Collaborative Research Inc., and tumor necrosis factor α from Immunex. AmpliTag DNA polymerase was obtained from Cetus, and poly(A)+ RNA, Moloney murine leukemia virus reverse transcriptase, and Sepharose beads were from Pharmacia LKB Biotechnology Inc. All other reagents were purchased from Sigma and were of the highest grade available.

**Cells**—The cells used were Flow 2000 embryonic fibroblasts (Flow Laboratories, Sydney, Australia) and HEL, human embryonic fibroblasts (Commonwealth Serum Laboratories, Melbourne, Australia). The cells were maintained in RPMI with 10% fetal calf serum and the Flow cells used between passage 17 and 19.

**Single Cell Isolation and PCR Analysis**—Individual Flow embryonic fibroblasts were harvested from subconfluence cultures with trypsin, diluted to low density, and single cells picked up with a drawn-out Pasteur pipette attached to a mouthpiece. The individual cells were transferred into microtiter wells and checked microscopically before being used for PCR analysis essentially as described by Rappolee et al. (18). RNA was prepared by guanidium thiocyanate extraction (19) and either used directly for PCR or stored at -70°C before use.

**Reverse transcription**—PCR was performed in a 50 μl reaction volume for 1 h at 37°C. Reverse transcription reaction mixture consisted of 5 μl of RNA (cell RNA and carrier tRNA), 50 mM Tris-HCl (pH 8.3), 75 mM potassium chloride, 10 mM dithiothreitol, 3 mM magnesium chloride, 500 μM each dATP, dTTP, dCTP, and dGTP (Pharmacia), 500 μg/ml oligo(dt), and 10,000 units/ml Moloney murine leukemia virus reverse transcriptase. On completion of the reverse transcriptase reaction, 5-μl aliquots were used in the PCR reactions. PCR were carried out in 100-μl reaction volumes. The cycle protocol was as follows: cycles 1–5: 95°C for 2 min, 55°C for 1 min, and 72°C for 1 min. Cycles 6–40: 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The PCR reaction mixture consisted of 10 mM Tris-HCl (pH 8.3), 50 mM potassium chloride, 1.5 mM magnesium chloride, 0.001% gelatin (according to Perkin-Elmer Cetus protocol for DNA amplification), 0.5 μl of cDNA, 1.25 mM dATP, dTTP, dCTP, and dGTP, 500 ng of primer 1, 1,500 ng of primer 2, and 0.5 μl of AmpliTag DNA polymerase. On completion of the PCR reaction, products were examined on a 6.5% acrylamide gel, eluted from the gel, and cut with appropriate restriction enzymes in order to confirm that the predicted DNA had been amplified.

The primers used were all 24-mers selected to encompass the transmembrane and cytoplasmic domains to the stop codon and were constructed using a Pharmacia Gene Assembler Plus. The primers used were as follows.

<table>
<thead>
<tr>
<th>Forward Primers</th>
<th>Reverse Primers</th>
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<tbody>
<tr>
<td>ACACAAGGAGGAGCCTCCCGGACCT</td>
<td>CACATTGGTTACCACTAATGTCAC</td>
</tr>
<tr>
<td>TGCTCATTGGCCTTTAGGGTTT</td>
<td>TGGCACTAATGTCAC</td>
</tr>
<tr>
<td>ATCATTGCTAGTAGCTAGCTT</td>
<td>ACGAGGAGGAGGAGCCTCCCGGACCT</td>
</tr>
<tr>
<td>TTCCTCGTGTGTTCTGGTAGCAC</td>
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**RESULTS**

**Individual Embryonic Fibroblasts Express Multiple Integrin β Chains in Association with αv**—Analysis of the surface phenotype of embryonic fibroblasts by flow cytometry after indirect immunofluorescent staining with a panel of monoclonal antibodies indicated that these cells expressed the αv subunit in considerable excess over the β3 subunit, whether
measured with polyclonal (rabbit) antibody to β3 or by a monoclonal antibody to the αvβ3 complex (Fig. 1A). In addition, the profile of fluorescence obtained (Fig. 1A) indicated that the great majority of cells within the population exhibited this staining pattern, rather than there being overlapping subpopulations of cells each with a different phenotype. Immunoprecipitates of αv from cells surface labeled with 125I-iodine were examined by nonreducing-reducing SDS-PAGE, and it was found that this α subunit was associated with a complex pattern of β chain polypeptides (Fig. 1B).

To determine the nature of the different αv-associated β chains, we performed a series of sequential immunoprecipitations with antibodies to different β subunits and analyzed the results by one-dimensional SDS-PAGE. In these immunodepletion experiments (Fig. 2), precipitation with an authentic antibody to β1 or to β3 prior to precipitation with the antibody mouse monoclonal antibody, and for the experiment shown under "Experimental Procedures." In the first precipitation (αv) one-half of the lysate was precipitated with a monoclonal antibody (13C2) to α chain of the vitronectin receptor. The remaining lysate was then precipitated two sequential times with an antibody to β1 (LM534), two sequential times with an antibody (SZ21) to β3, then with the antibody to αv. Each of the immunoprecipitates was then analyzed on a 7.5% SDS-polyacrylamide gel under nonreducing conditions, and the autoradiograms are illustrated. Relative molecular masses (Mr) are shown to the left.

![Fig. 1. The αv integrin subunit is expressed in excess over the β3 subunit and is associated with multiple β chains on the cell surface.](image)

![Fig. 2. Sequential immunoprecipitation identified β1 and β3 subunits in association with the α chain of the vitronectin receptor (αv).](image)
Multiple Integrin β Chains

Fig. 3. Sequential immunoprecipitation and \(^{32}P\) labeling identified β5 in association with αV. Detergent extracts of surface-iodinated Flow embryonic fibroblasts were sequentially precipitated three times with an antibody (23C6) to the αvβ3 heterodimeric complex (Sequential, tracks 1–3) then with an antibody (13C2) to the α chain of the vitronectin receptor (Sequential, αω). The track labeled αω and \(^{32}P\) is the immunoprecipitate obtained from the same cells biosynthetically labeled with \(^{32}P\), stimulated briefly with phorbol ester, and immunoprecipitated with an antibody (13C2) to αω as described under “Experimental Procedures.” The immunoprecipitates were analyzed by nonreducing SDS-PAGE on 7.5% gels, and the autoradiograms are shown. The arrowheads indicate the relative positions of the integrin subunits, αω, β1, and β5.

Fig. 4. PCR analysis of integrin subunit mRNA from two clones of Flow embryonic fibroblasts. Oligonucleotide primers spanning the cytoplasmic/transmembrane domains of the integrin subunits indicated were used to amplify cDNA prepared from mRNA isolated from two separate cells from the Flow 2000 embryonic fibroblast line. The expected sizes of the mRNA amplified products are: αω, 250 bp; β1, 300 bp; β3, 200 bp; β5, 250 bp. Molecular weight markers are shown in base pairs.

and β5 were expanded by PCR using primers specific for each integrin subunit. The identity of the individual bands obtained with each pair of primers was confirmed by digestion with restriction enzymes and re-running the samples to show cleavage to fragments of the appropriate predicted size. Exclusion of genomic DNA as a source of the PCR fragments was made in control experiments using isolated nuclear DNA (1 μg) treated with RNase. Eight individual cells were examined by PCR (Fig. 4 and Table I), and it was found that all expressed mRNA for αω, β1, and β5, and six had mRNA coding for β5. It is of interest that from all of the clones, the β5 primers yielded two bands by PCR (Fig. 4), the smaller of which identified mRNA of the size reported for β5 when tested by Northern blot analysis, and upon sequencing the PCR product was identical to the published β5 sequence (data not shown). The larger band also illuminated this mRNA in Northern blots but identified in addition a larger species of mRNA. To date we have found the additional PCR fragment in two different lines of embryonic fibroblasts (Flow 2000 and HEL) but not in adult colonic fibroblasts which express mRNA for β5 (CCD 18); it is possible that it represents an alternatively spliced form of β5 restricted to embryonic cells, as alternatively spliced forms of other integrins, including β3, have been reported by others (16). If this is the case, and all of the different mRNA are translated, these data suggest that individual embryonic fibroblasts can simultaneously display β1, β3, β5, and β5'.

Regulation of the Surface Expression of Integrin β Chains Associated with αv—Repeated immunoprecipitation analysis of the Flow 2000 fibroblasts over a period of time sometimes showed a preponderance of αvβ1 and αvβ5 over αvβ3 on the cell surface, and on other occasions the major αv-associated integrin was αvβ3 (data not shown). With continuous passage this line mainly displayed αvβ1, β5, whereas another line of embryonic fibroblasts (HEL) expressed αvβ3 as its major αv-associated integrin, and despite the presence of abundant β1 integrin, very little β1 was found in association with αv (see Fig. 2 above). These data suggested to us that the relative proportions of the different β chains that associate with αv might be regulated by different conditions of cell culture or by autocrines.

To determine whether the proportions of the different αv-associated β chains could be manipulated, cell cultures were treated with a range of cytokines known to modulate biological activity, then analyzed for their surface expression of αv-associated integrins. In short term assays (6 h of culture), platelet-derived growth factor and particularly tumor necrosis factor α (TNFa) up-regulated the surface expression of αv and all of the associated β chains, but there appeared to be no change in the relative proportions of the different β chains (Fig. 5), and transforming growth factor β1 (TGF-β1) appeared to have no effect over this short time period. Cells cultured overnight in the presence of TNFa showed a slight increase in expression of all of the integrin subunits (data not shown), and those cultured in basic fibroblast growth factor (FGFb) showed a decrease in β1 and β5 expression with no reduction in the amount of β3 associated with the αv subunit.

Much more dramatic was the effect of the culture conditions themselves. The foregoing experiments were carried out with cells grown to subconfluence. When the cells were enabled to grow to complete confluence, analysis of their surface phenotype showed a total loss of β3 expression, although the cells continued to display αvβ1 and αvβ5 (Fig. 6). This absence of β3 expression was maintained when the confluent cells were cultured overnight in the presence of TNFa or FGFb; as with the subconfluent cells, the levels of αv-associated integrin expressed appeared to increase slightly with TNFa, but the presence of FGFb did not induce any β3 expression (Fig. 6). Immunoprecipitations from these confluent cultures also re-

\begin{table}
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\caption{PCR analysis of integrin subunits from individual clones}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Integrin subunit} & \textbf{αω} & \textbf{β1} & \textbf{β3} & \textbf{β5} & \textbf{β5'} & \\
\hline
\textbf{No. of cells that gave a positive PCR result} & 8 & 6 & 8 & 8 & 8 & 8 \\
\hline
\end{tabular}
\end{table}

\footnote{L. M. Rankin and G. F. Burns, unpublished data.}
vealed much more clearly the presence of an additional αv-associated β chain (Fig. 6) seen only faintly or not at all in the subconfluent cultures (Fig. 5). We have not yet identified this unknown β chain (labeled βu in Fig. 6), but its relatively greater migration in SDS-PAGE and its small shift in apparent molecular weight upon reduction together with its non-reactivity with polyclonal antibodies to β3 discriminates it from β1, β2, and β5.

**αvβ5 but Not αvβ1 Binds to Vitronectin**—The αvβ1 combination has been described as a receptor for vitronectin (12) or fibronectin (11) on different cell types, and αvβ5 has been shown to bind vitronectin alone (7, 9) or vitronectin and fibronectin (6). Other integrins have been demonstrated to exhibit different ligand binding depending on their cell of origin (17) and it is possible that the different reports on the function of αvβ1 reflect the different cell types used (11, 12). The fact that the embryonic fibroblasts expressed both αvβ1 and αvβ5 enabled us to compare directly the capacity of these integrins (from the same cell) to bind to vitronectin. To simplify the interpretation and to maximize the amount of cell surface receptor available, the fibroblasts were grown to confluence and treated overnight with TNFα before carrying out affinity chromatography and immuneprecipitation with the cell lysate. From the results (Fig. 7) it was apparent the αvβ5 bound to the vitronectin column whereas αvβ1 could not be detected in the column eluate. As with the immunoprecipitation experiments described above, no αvβ3 was detectable from these confluent cells. In experiments using fibronectin as the affinity ligand it was found that αvβ3 bound to this column, but αvβ5 did not bind in detectable amounts (data not shown). The βu subunit was not seen in precipitations from either column (Fig. 7 and data not shown).

**RESULTS**

It is now clear from a number of studies that the integrin αv subunit can form associations with multiple β chains including β1, β3, β5, β3b (5–12), and probably other, as yet uncharacterized β subunits (this report and discussed in Ref. 11). At least some of the receptors thus formed bind to the same matrix protein (5–8, 12), and the question arises as to why such apparent redundancy should occur. Since integrin β chains may mediate transmembrane signals (28, 29) it is now clear from a number of studies that the integrin αv subunit can form associations with multiple β chains including β1, β3, β5, β3b (5–12), and probably other, as yet uncharacterized β subunits (this report and discussed in Ref. 11). At least some of the receptors thus formed bind to the same matrix protein (5–8, 12), and the question arises as to why such apparent redundancy should occur. Since integrin β chains mediate transmembrane signals (28, 29) we have suggested that the association of one α chain with multiple β chains would enable different signals to be transduced in response to a single matrix protein (8). Certainly a number of cell types preferentially or exclusively express a single β chain (8, 10–12, 21). However, other cell populations express multiple β chains that can associate with αv (8, 10, 11, 14), and if it could be shown that individual cells within the population displayed multiple β chains then this proposal would be less attractive. Here, we show that this is indeed the case, since individual embryonic fibroblasts expressed mRNA for β1, β3, and β5 as well as for αv.

The results obtained by PCR could not be confirmed as protein data for single cells, since antibodies to β5 were not available for the present study. However, flow cytometry indicated that the population of fibroblasts under study ap-
FIG. 7. Analysis of the αv-associated integrins from Flow embryonic fibroblasts that bind vitronectin. Flow embryonic fibroblasts were grown to complete confluence and then treated overnight with TNFα (10 units/ml). The cells were surface-iodinated, washed, and applied to a 1-ml affinity column of vitronectin equilibrated in the same buffer. The column was washed and the bound proteins eluted with GRGDSP peptide and 300 mM sodium chloride as described under “Experimental Procedures.” The eluate was dialyzed overnight against “complete” octyl glucoside buffer, one-half of the integrin bands (bound, αv) were immunoprecipitated with a monoclonal antibody to the αv subunit (13C2), and the other fraction sequentially immunoprecipitated two times with a monoclonal antibody to the β3 subunit (Z21), and then with the anti-αv antibody. The precipitates were run (nonreduced) on SDS-polyacrylamide gels (7.5%) and subjected to autoradiography. Also shown is a sample of the fraction that did not bind to the column (full-through, FT; equivalent counts to precipitates) and a sample of the affinity beads boiled in SDS-PAGE sample buffer after elution of the integrin bands (bound, βD).

peared to be fairly homogeneous with all of the cells expressing levels of αv greatly in excess of β3 or of the αvβ3 complex (Fig. 1). Surface labeling and sequential immunoprecipitation studies revealed the presence of the β1 and β3 subunits in association with αv, together with another band that could be identified as β5 because it was rapidly and strongly phosphorylated in response to treatment of the cells with phorbol ester (7). Also, other than platelets and their progenitors, neither β3 or β5 are known to associate with any α chain other than αv (9). Hence it seems reasonable to deduce that individual cells are capable of coexpressing multiple β chains in association with αv on their cell surface. These cells may also be expressing additional β subunits, at least one of which is in association with αv (Fig. 6). In the results obtained by PCR we obtained a higher band (∼350 bp) in addition to the band at 250 bp expected with the β5 primers used. This band appeared to be a fragment of a real product, since when used in Northern blot analysis it hybridized with a band at 3.6 kilobases in addition to the β5 band at 3.3 kilobases.3 McLean et al. (14) carried out PCR reactions with β5 primers on a number of cell types and never detected an additional band, nor did they detect more than a single species of mRNA in hybridization experiments. These authors (14) did not specifically state the oligonucleotide primers that they used, but one was immediately following the termination codon, and the other was 5′ in the coding region encompassing the transmembrane and/or cytoplasmic domains. Since the expected size of their amplified fragment was 250 bp (as ours) and encompassed the same regions as ours, it would appear that these differences are not due to different primers being used. We consistently identified the additional band by PCR from two different lines of embryonic fibroblasts but not from any of the other cell lines studied including adult colon-derived fibroblasts.3 There have been described alternatively spliced forms of β3 and β4 (16, 30), both contained within the cytoplasmic region, although the significance of these is not known. If it can be confirmed that the β5′ identified in the present report is an alternatively spliced form of β5, its restricted tissue distribution may point to interesting biological consequences. How this β5′ product relates to the additional β polypeptide (βu) associated with αv on the surface of confluent cells (Fig. 6) is not known, although the product of β5′ might be expected to be larger rather than smaller than β5.

The presence of multiple β chains in association with αv on the same cells enabled us to examine possible regulators of integrin expression, seeking in particular to identify agents that altered the ratios of the different αvβ subsets. In studies of the regulation of integrin expression by TGF-β1, Massague and colleagues (31, 32) demonstrated that whereas cells treated with TGF-β1 generally increased their level of integrin expression, such treatment did not alter the different α chains shown to associate with β1 in the fibroblasts under study. We tested a range of potential mediators, including TGF-β1, for their ability to influence the surface expression of αv-associated β chains, and although some changes in the overall levels of αvβ integrin were observed (in particular a significant increase upon treatment with TNF-α) only FGFβ caused any change in the proportion of β chains that were expressed. Overnight culture with this growth factor caused the cells to exhibit predominantly αvβ5 because of an apparent loss of αv-associated cell surface β1 and β5. When the embryonic fibroblasts were grown to confluence, they totally lost expression of β3 but continued to express the other αvβ integrins to the same level, and αvβ3 to an increased level. Freed et al. (7) discussed their impression that MG-63 osteosarcoma cells expressed more β3 (β5′) and less β3 with time of passaging in tissue culture. We have come to a similar conclusion regarding the Flow embryonic fibroblasts used predominantly in the present report; over some 20 months of study, using different but progressively older subcultures, these cells have begun to exhibit relatively increased αvβ1 and αvβ5 and less αvβ3 than we observed in early experiments. Certainly it is quite striking to compare a young culture of human embryonic lung fibroblasts (passage 17) with the Flow cells in passage 26 (see Fig. 2) wherein the younger cells express predominantly αvβ3. Therefore, it can be postulated that senescence and a slowing of growth, perhaps caused by senescence or imposed by growth restrictions, accompany or induce β chain switching, with αvβ3 being expressed predominantly by actively dividing cells. Other unidentified variables may also play a part, and our own data with a number of melanoma cell lines (21), many of which express only αvβ3, contrasts with reports of other cell types expressing only αvβ1 or αvβ5 (6, 11). In this regard it is interesting to note that the VLA (β1) integrin subfamily appears to behave in a similar manner, whereby serum deple-
human embryonic fibroblasts does not bind to vitronectin, whereas avp35 from the same cells does so. Both our own study and that of Vogel et al. (11) used column affinity chromatography to demonstrate receptor binding, whereas other studies had relied upon whole cell attachment assays and antibody-mediated inhibition (12). In this regard it is noteworthy that Cheresh et al. (6) initially provided data from whole cell attachment assays suggesting that the avp35 receptor bound to both vitronectin and fibronectin, whereas subsequent studies by the same group using purified receptor protein indicated that avp35 bound only to vitronectin (9). It may be, therefore, that the intact cell is able to utilize individual receptors for different functions, perhaps in association with accessory molecules not present in the purified protein. On the other hand, caution must be exercised when intact cells are employed, since clearly there are other integrin and non-integrin receptors for many of these matrix proteins, including fibronectin (34, 35). By whatever measurement, however, there is general agreement that neither avp31 nor avp35 bind to the complete range of ligands recognized by avp33 (5), and we could not detect avp3a binding to either vitronectin (Fig. 7) or fibronectin. Hence it appears that embryonic fibroblast av-associ-ated integrin expression may progress from one general purpose complex (avp3) to more specific individual receptors.

The present study has concentrated upon the surface expression of the av-associated integrin receptors. In this way we have been able to delineate the different β chains associating with αv, their ligand specificity, and conditions that influence their expression. An understanding of what regulates the assembly and expression of the different αv-associated β subunits will require detailed study of the relative rates of transcription, protein synthesis, and subunit association, and in describing some of the conditions that influence the relative surface expression of the different subunits our results will provide a useful starting point for such studies.

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REFERENCES