The laminin-binding \( \alpha 7 \beta 1 \) integrin receptor is expressed at high levels by skeletal and cardiac muscles and by certain melanocytic cells. We have assessed the potential role of the \( \alpha 7 \beta A/B \) integrin isoforms in mediating cell adhesion and motility and determined the laminin isoform specificity of this integrin. When MCF-7 breast carcinoma cells, normally nonadherent to laminin 1, were stably transfected with cDNA for mouse \( \alpha 7 \), they adhered with high efficiency and migrated on laminin 1 substrates. Function-perturbing monoclonal antibodies generated to mouse \( \alpha 7 \) subunit blocked both adhesion and migration of \( \alpha 7 \) transfectants on laminin 1 substrates. Additional studies with MCF-7 transfectants revealed that \( \alpha 7 \beta 1 \) binds well to laminin 1 and to a mixture of laminin 2 and 4 but not to laminin 5. Importantly, \( \alpha 7 \beta 1 \) was capable of promoting motility on both laminin 1 and laminin 2/4 substrates. However, MCF-7 cells transfected with cDNA for either \( \alpha 7 A \) or \( \alpha 7 B \) showed no significant differences in cell adhesion or motility on laminin 1 substrates. Although the role for the alternatively spliced cytoplasmic variants of \( \alpha 7 \) remains unknown, the results establish that \( \alpha 7 \beta 1 \) mediates cell adhesive activities on a restricted number of laminin isoforms.

Laminins are adhesive glycoproteins found in basement membranes that promote diverse cellular responses. Cell adherence to laminin matrices plays an important role in maintaining normal tissue organization and in tissue renewal and repair. The interaction of cells with extracellular matrix macromolecules like laminins is mediated primarily by heterodimeric receptors from the integrin superfamily (reviewed in Ref. 1). Integrins provide linkage between the component elements of the extracellular matrix and the structural constituents inside the cell. Besides serving as adhesion receptors, integrins can transmit signals from the extracellular matrix to the cell interior that can activate several pathways, ultimately influencing an array of cellular properties including proliferation, differentiation, survival, and apoptosis (2).

Laminin 1 was the first fully characterized isoform and is composed of \( \alpha 1, \beta 1 \), and \( \gamma 1 \) chains. This prototypic laminin is a large molecular weight trimer with multiple domains that are involved in cell adhesion and interactions with other basement membrane components such as nidogen, type IV collagen, and heparan sulfate proteoglycan (3). Several other laminin isoforms have been identified and include laminin 2 (\( \alpha 2 \beta 1 \gamma 1 \), merosin), laminin 3 (\( \alpha 1 \beta 2 \gamma 1 \), S-laminin), laminin 4 (\( \alpha 2 \beta 2 \gamma 1 \), S-merosin), laminin 5 (\( \alpha 3 \beta 3 \gamma 2 \), calinin/nicein/epiligrin), and less well characterized laminins 6–10 (reviewed in Refs. 3–5). The different laminin isoforms are tissue-specific and are expressed in a developmentally regulated pattern. Laminin 1 contains multiple sites for integrin-mediated cell attachment, which is effectuated by several \( \beta 1 \) integrins (5). Of these \( \alpha 3 \beta 1 \), \( \alpha 6 \beta 1 \), and \( \alpha 7 \beta 1 \) bind to the long arm fragment \( \epsilon 8 \) of laminin, produced by elastase digestion. The \( \alpha 3 \beta 1 \) and \( \alpha 2 \beta 1 \) integrins, which both contain the I domain, bind to the cross-region of laminin represented by the short arm fragment.

The biological response to laminin appears to be cell type-specific, and this may be due in part to the specific integrin receptors expressed by individual cells. \( \alpha 7 \beta 1 \), originally found in melanoma cells, is a muscle-specific integrin (6) that binds to laminin (7–9). Although there is only one \( \alpha 7 \) gene, complicated splicing mechanisms result in several \( \alpha 7 \) isoforms. Several studies have shown that alternative splicing generates two isoform subsets: (i) X1 and X2 and (ii) A, B, and C, which differ at extracellular and cytoplasmic regions, respectively (10–13). Upon terminal differentiation of myoblasts, isoform switching and up-regulation of \( \alpha 7 \) expression are detected. The extracellular variants have altered sequence in the ligand binding domain and may have different laminin isoform specificity or affinity. The cytoplasmic isoforms, which share the common extracellular and transmembrane domains but differ at the cytoplasmic region, may trigger different biological functions when cells interact with laminin.

In the present study, we have stably transfected MCF-7 carcinoma cells, which normally do not adhere to laminin 1, with mouse \( \alpha 7 \) cDNA. We also generated function-perturbing monoclonal antibodies to mouse \( \alpha 7 \) integrin to inhibit \( \alpha 7 \)-extracellular matrix interactions. Using these approaches, we demonstrated that both \( \alpha 7 A \) and \( \alpha 7 B \) mediate adhesion and migration of MCF-7 transfectants on laminin 1 and laminin 2/4 substrates; however, \( \alpha 7 \beta 1 \) does not bind to laminin 5.

**EXPERIMENTAL PROCEDURES**

**Materials**—The human breast carcinoma cell line MCF-7 was from American Type Culture Collection and was grown in Dulbecco’s modified Eagle’s medium H-16 with 10% fetal bovine serum. Laminin 1 was purified from mouse Engelbreth-Holm-Swarm tumor as described previously (14). Human placental laminin was purchased from Life Technologies, Inc. and is known to be a mixture of laminin 2 and 4 (14, 15). Purified human laminin 5 was kindly provided by Dr. Robert Burgeson (Cutaneous Biology Research Center, Boston, MA). Human plasma fibronectin was purchased from Collaborative Biomedical Products (Bedford, MA). Antibodies against integrin subunits included the rat anti-human \( \beta 1 \) mAb \( A 2 B 2 \) and rat anti-human \( \alpha 5 \) mAb \( B 9 2 2 \), kindly provided by Dr. Chung-Chen Yao, Barry L. Ziober, Rachel M. Squillace, and Randall H. Kramer‡

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The abbreviations used are: mAb, monoclonal antibody; PBS, phosphate-buffered saline; BSA, bovine serum albumin.
Caroline Damsky (University of California, San Francisco, CA); mouse anti- 
CD235a (Serotec, North Oxford, UK), and polyclonal antibody to the 
type 114, expresses moderate levels of 
a subunit in clone 114; as expected for this integrin under non-
reducing conditions. We transfected cDNA encoding 
a7B subunits into MCF-7 cells. MCF-7 cells nor-
maingly adhere poorly to laminin 1 (17). At least 10 clones of each 
transfectant were isolated and characterized, and several high 
expressing clones were obtained for both a7A and a7B. High 
expressers for a7A (clone 114) and a7B (clone G) were chosen 
for further analysis. Fluorescence-activated cell sorting analy-
sis with mAbs against different laminin-binding integrins showed 
that one of the high a7A-expressing cell lines, clone 114, 
expresses moderate levels of a2, a3, and a6 and high 
amounts of a7 that correspond to means of fluorescence inten-
sity of 84.8, 34.1, 35.9, and 645.3, respectively (Fig. 1A). For 
the parental MCF-7, a2 (104.1) and a6 (25.4) levels were similar, 
but a3 was expressed at a mean fluorescence intensity of 67.0. 
We also found that the β1 integrin level was increased in clone 
114 in compensation to the increased level of a7 on the cell 
surface (data not shown).

The immunoprecipitation analysis of surface biotinylated 
parental MCF-7 cells and clone 114 cells verified specificity of 
the mAb and in addition showed that a7β1 integrin was 
expressed in transfectants but was not detectable in the parental cells (Fig. 1B). Immunoprecipitation of cell lysates with CY8, a mAb against the extracellular domain of a7, yielded the a7 subunit in clone 114; as expected for this integrin under 
nonreducing conditions, the β1 subunit partner comigrated with the 
a7 subunit (Fig. 1B, lane 3) (7, 18). Following reduction, the 
β1 subunit exhibited decreased mobility, whereas the a7 sub-
unit was cleaved to yield a 100-kDa fragment and an ~30-kDa 
fragment containing the cytoplasmic tail (Fig. 1B, lane 3). The 
a7β1 bands were also detected by using polyclonal antibody 
22780 to a7A and polyclonal antibody 1211 to a7B in clone 114 
and clone G, respectively (data not shown). In other studies, 
we have found that transfection of MCF-7 cells with cDNA of a6 
integrin leads to significant expression of the α6β4 complex.3 In 
the case of MCF-7 cells transfected with a7, the integrin does
Human placental merosin contains primarily laminin 4 (α2β2γ1, S-merosin) with lesser amounts of laminin 2 (α2β1γ1, merosin) (14, 15); the laminin 5 preparation, isolated from conditioned medium of human keratinoctyes, consists of laminin chains αβ3γ2 (19). Both parental MCF-7 cells and α7 transfectants adhered to laminin 2/4. However, analysis of adhesion to this mixture of laminins is complicated by the presence of α2, α3, and α6 integrins. The adhesion of both parental and α7-transfected cells to laminin 2/4 was partially blocked by anti-α2 mAb (VM1) and anti-α3 mAb (P1B5). The combination of function-perturbing mAbs anti-α2 (VM1), anti-α3 (P1B5), and anti-α6 (GoH3) completely blocked attachment of parental cells but only partially inhibited attachment of the α7 transfectants (Fig. 2C). Interestingly, α7-transflectant adhesion to laminin 2/4 was inhibited by nearly 80% with α7-perturbing mAb CY8; and the combination of anti-α2 (VM1), anti-α3 (P1B5), and anti-α6 (GoH3) and anti-α7 (CY8) completely blocked the adhesion of α7-transfectants to laminin 2/4 substrates. Thus, α7 can clearly bind to and mediate adhesion to laminin 2/4.

In adhesion assays with laminin 5, we used as a positive control a human squamous carcinoma cell line (HSC-3) that binds strongly to laminin 5 via the α3 integrin, which is highly expressed in these cells (16). At laminin 5 concentrations from 0.3 to 3 μg/ml, HSC-3 cells showed a dose-dependent increase in adhesion, whereas MCF-7 parental cells showed only moderate binding efficiency (Fig. 3A). However, the α7-transfected clone 114 cells bound poorly to the laminin 5 substrate. Under the same assay conditions, HSC-3 and clone 114 cells adhered efficiently to laminin 1 in a dose-dependent fashion, whereas the MCF-7 parental cells did not (Fig. 3B). In other studies with MCF-7 parental cells and α7 transfectants, adhesion to laminin 5 could be totally blocked with mAb against α3 (data not shown). As mentioned above, analysis of integrin profile indicates that in the α7-transfected clone 114 cells, α3β1 levels are decreased ~50% compared with that of the parental cell population. Thus, there is a correlation between adhesion to laminin 5 and expression of α3. This clearly demonstrates that α7 integrin in MCF-7 cells binds to laminin 1 and laminin 2/4 but cannot efficiently mediate binding to laminin 5.

Laminin Induces Motility in α7 Transfectants—We next examined the locomotive response of parental MCF-7 cells and α7 transfectants to laminin 1 (Fig. 4, A and B) and laminin 2/4 (Fig. 4C) substrates in a modified Boyden chamber assay. The parental MCF-7 cells are known to be poorly migratory on laminin 1 (20), and several growth factors have been shown to stimulate their motility on other ligands (21, 22). However, both of the α7-transfected clones (114 and G) showed an enhanced motile response on laminin 1 compared with the parental cells; furthermore, in the presence of basic fibroblast growth factor (1 ng/ml) as a stimulant the motile response was enhanced. These results established that transfection of α7A or α7B is sufficient to convert MCF-7 cells into migratory cells on laminin 1. In addition, motility of clone 114 cells on this substrate was completely blocked by CY8 monoclonal antibody to α7 (Fig. 4B).

On laminin 2/4, both parental MCF-7 cells and clone 114 cells were motile (Fig. 4C). Function-perturbing antibody to α7 blocked the motility of clone 114 cells by more than 50%. In the presence of a combination of function-perturbing antibodies to α2, α3, and α6, migration of parental MCF-7 cells was completely inhibited, but there was little effect on clone 114 cells (Fig. 4C). Finally the combination of anti-α2, -α3, -α6, and -α7 mAb or anti-β1 completely blocked the motility of both parental and clone 114 cells. These results clearly show that the α7
integrin binds and promotes motility on laminin 1 and laminin 2/4 substrates.

**DISCUSSION**

In this study, we have demonstrated that the α7β1 integrin can mediate adhesion and migration on a restricted number of laminin isoforms. We used two approaches successfully: (i) a gain of function approach by transfecting α7 cDNA into MCF-7 cells and (ii) a loss of function approach by using function-perturbing antibodies against α7 on cells expressing this integrin. Evidence obtained from these approaches clearly shows that exogenously expressed α7 confers on MCF-7 cells both the ability to bind and the ability to migrate on laminins. While this work was in preparation, Echtermeyer et al. (9) reported that transfection of mouse α7 cDNA into the human 293 embryonic kidney cells conferred a motile phenotype on laminin 1. Interestingly, this enhancement in motility occurred even α7 transfectants adhere poorly to laminin 5. Dose response study of cell adhesion to laminin 5 (A) and laminin 1 (B). HSC-3, a human squamous carcinoma cell line, was used as a positive control. On laminin 5, HSC-3 and MCF-7 cells showed increases in adhesion that paralleled increasing coating concentration. However, the α7-expressing clone 114 cells adhered poorly to laminin 5 substrate. On laminin 1 substrate, HSC-3 and clone 114 cells showed a strong dose-dependent increase in adhesion with increasing coating concentration while MCF-7 cells adhered poorly. ○, MCF-7; ●, clone 114; ×, HSC-3.
though the parental cells expressed additional laminin 1-binding receptors.

We examined the ligand specificity of the a7 receptor using available laminin isoforms. In MCF-7 a7 transfectants, a7B1 mediated binding to preparations of laminin 1 and to human placental laminins (a mixture of laminin 2 and 4). In contrast, laminin 5 was a poor substrate for a7-expressing cells. Eventually, when pure preparations are available, the functions of a7 should be tested on additional members of the laminin superfamily, especially laminin 2 (merosin), which is present in the basement membrane surrounding adult skeletal myofibers where a7 normally is detected. It is interesting that another laminin-binding integrin, a6B1, sharing high amino acid sequence homology with a7, not only binds to laminin 1 and human placental merosins (laminin 2 and 4) but also binds efficiently to laminin 5 (23). In addition, a6 can pair with B1 or B4 subunit, whereas as we show here in the MCF-7 transfectants, a7 pairs only with B1 subunit. Thus, even though a6 and a7 share strong amino acid sequence identity, there must be distinct domains that define both ligand specificity and pairing preferences. In the parental MCF-7 or transfectants, moderate levels of a2, a3, and a6 are expressed, yet these integrins are not capable of mediating adhesion or migration on laminin 1. It appears that this set of integrins, in contrast to a7, is not constitutively active for laminin 1. However, on laminin 2/4, this same set of integrins in the parental MCF-7 cells can mediate both adhesion and motility, yet in the transfectants is not fully competent for these adhesive interactions.

It is interesting that a7 transfection appeared to decrease the ability of existing integrins on the MCF-7 cells to interact with laminin 2/4 and with laminin 5. One possibility for the decrease in the activity of integrins a2 and a3 is due to a decrease in their expression. Fluorescence-activated cell sorting analysis confirms that the high expression of a7 caused a modest decrease in the expression level of a3. Another contributing factor may be that the high a7 expression produces a dominant negative effect that down-regulates the activity of the other integrins. Studies have suggested that certain integrins can produce a modulating effect on the function of other integrins. For example, in lymphocytes activation of LFA-1 can down-regulate a4 activity (24). A different phenomenon is observed in the a5-deficient CHO cells where full activity of a5B3 receptor requires the presence of transfected a5 integrin (25). Thus an integrin may induce down-regulation of another integrin’s function, or alternatively two integrins may cooperate with each other to modulate function.

It is conceivable that the cytoplasmic variants of a7 function differently. RNA alternative splicing events in the a cytoplasmic region have been detected in several integrin molecules, including a3, a6, and a7 (10–12, 26–29). However, functional significance of the a chain-cytoplasmic isoforms has not been well established. Results from recent studies searching for functional differences between a6A and a6B are still controversial (23, 30–33). Our results indicate that a7A and a7B receptors are equally active in their adhesive or migratory activities. It is possible that a7A/B, a muscle-specific integrin, will show differential activities only in the context of a muscle-specific environment.

In summary, the results presented here show that a7B1 can mediate both cell adhesion and migration on laminin 1 and laminin 2/4. Importantly, we have demonstrated that a7 can interact with these different laminin substrates but not with epithelial cell-specific laminin 5. These results strongly support the role of the a7 receptor in mediating interactions with specific laminin isoforms. The tissue-specific expression of different family members of integrins and laminins (e.g. a7B1 and merosins in skeletal muscle (13, 34)) suggests that there is a selective interaction that may be important in both embryonic development and tissue homeostasis.

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