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INTEGRIN DEPENDENCE OF CALU-1 CELL MOTILITY ON ENDOTHELIAL EXTRACELLULAR MATRIX PROTEINS

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Abstract

We recently showed that the motility of the malignant human epidermoid lung carcinoma cells Calu-1 correlates with their expression levels of $\alpha 2$, $\alpha 3$, $\alpha 6$, and $\beta 1$ integrin subunits. To determine a causative relationship underlying this correlation, here we measured Calu-1 cell adhesion to and migration on laminin, collagen IV, human umbilical vein endothelial cell monolayers, and endothelial cell extracellular matrix in the presence of function-blocking antibodies against the suspect integrin subunits. Blocking individual α subunits did not affect adhesion to or motility on laminin, but when used in pair-wise combinations, monoclonal antibody treatments significantly decreased tumor cell motility on, without diminishing adhesion to, laminin and the other substrates. Blocking all three α subunits at once or the $\beta 1$ subunit alone abolished migration on laminin; however, the latter treatment also abolished adhesion, whereas the former treatment did not. By contrast, blocking the $\beta 1$ subunit significantly reduced motility on collagen IV, endothelial cell monolayers, and endothelial cell extracellular matrix, but always without affecting adhesion. These results suggest a separation of roles and mechanisms of different integrins in adhesion and motility.

Key terms

tumor cell motility; cell adhesion; Integrin; Persistent random walk; extracellular matrix

Introduction

Tumor cell motility plays a vital role in the spread of cancer, as it is required for successful metastasis to occur. In blood borne metastasis, tumor cells are believed to have acquired an increased ability to migrate across the barriers of the endothelial cell and basement membrane layers. Using a modified Boyden Chamber assay, we have previously shown that malignant Calu-1 epithelial lung carcinoma cells are better able to migrate across a human umbilical vein endothelial cell (HUVEC) monolayer than are non-malignant L132 lung epithelial cells⁴. This enabled us to develop the following working hypothesis: Tumor cells, via increased expression of cell surface receptors, are capable of opportunistic use of factors presented in their journey of dissemination to upregulate their motility, thereby migrating through any barriers such as

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endothelial monolayers and basement membranes. In a recent paper, we conducted initial testing of this hypothesis.

To narrow down the possible sources of such migration-stimulating factors we used individual cell track analysis to show that, of the sources tested, the extracellular matrix (ECM) secreted by the HUVECs themselves upregulated motility of Calu-1 cells but not L132 cells, while other sources, including soluble factors, did not¹³. Further investigation of the effects of individual ECM proteins revealed that Calu-1 motility was stimulated by collagen I, collagen IV, fibronectin, or laminin, while L132 motility was not. L132 motility was greater than Calu-1 motility only on vitronectin. These results led us to focus on the integrins, a class of known receptors for these ECM proteins. Flow cytometric analysis revealed that only integrin subunits $\alpha 2$, $\alpha 3$, $\alpha 6$, and $\beta 1$ are expressed at substantially higher levels on the Calu-1 cells than on the L132 cells. Other integrins were found to have comparable or low expression levels on both cell lines. These data support the hypothesis that integrins $\alpha 2\beta 1$, $\alpha 3\beta 1$, and $\alpha 6\beta 1$ are responsible for the better ability of malignant Calu-1 cells than non-malignant L132 cells to migrate through HUVEC monolayers. Here we present results of the next step of the investigation, which was directed towards establishing a mechanistic causative relationship for the phenomenologic correlation.

To determine if the higher expression of integrin subunits $\alpha 2$, $\alpha 3$, $\alpha 6$, and $\beta 1$ is indeed responsible for the increased Calu-1 mobility, the effects of function-blocking monoclonal antibodies (mAbs) directed against these integrin subunits on Calu-1 adhesion to and migration on their ligands, isolated ECM proteins collagen IV and laminin were measured. Since the goal of this investigation was to elucidate mechanisms enabling Calu-1 cells to have increased invasiveness over L132 cells, the dependence of the adhesion and motility of the Calu-1 cells in the more physiologic cases – on HUVEC ECM and HUVEC monolayers – on the suspect integrins was also measured.

Calu-1 cell motility was found to strongly depend on $\beta 1$ integrins. Blocking individual α subunits did not affect motility, but using a combination of mAbs against the three target α subunits decreased Calu-1 cell motility. Calu-1 motility, but not adhesion, was adversely affected by the function-blocking antibodies except for the anti- $\beta 1$ mAb, which affected adhesion to laminin in addition to blocking motility. These results suggest a separation of functionality between cell surface receptors used by the cells for adhesion and motility.

Materials and Methods

Cells and Antibodies

Human umbilical vein endothelial cells (HUVECs) obtained from Clonetics (Walkersville, MD) and human lung epidermoid carcinoma Calu-1 cells obtained from American Type Culture Collection (Rockville, MD) were cultured according to standard practices as described previously¹³.

Dulbecco's Modified Eagle's Medium (DMEM), Phosphate Buffered Saline (PBS), Disodium Ethylenediamine Tetraacetate (EDTA), Bovine Serum Albumin (BSA), Sodium Dodecyl Sulfate (SDS), and other cell culture reagents were obtained from Sigma Chemicals (St. Louis, MO). Fetal Bovine Serum (FBS) was obtained from Gibco BRL Life Technologies (Grand Island, NY). EBM-2 medium was obtained from Clonetics. $^{51}\text{Cr} - \text{Na}_2\text{CrO}_4$ in normal saline – was obtained from ICN Pharmaceuticals (Irvine, CA). Function-blocking mAbs directed against $\alpha 2$ (P1E6), $\alpha 3$ (P1B5) were from Chemicon International (Temecula, CA). The anti- $\alpha 6$ (GoH3) and anti- $\beta 1$ (P4C10) mAbs were respectively from Beckman Coulter (Fullerton, CA) and Gibco. In both cell adhesion and cell motility assays described below, anti- $\alpha 2$, anti- $\alpha 3$, and anti- $\alpha 6$ mAbs were used at a final concentration of 10 $\mu\text{g}/\text{ml}$ after confirming this to

be a saturating concentration by flow cytometry analysis (data not shown). Anti- $\beta 1$ mAb in ascites was used at various dilutions.

Extracellular Matrix Protein Preparation and Coating

24- and 48-well plates were prepared with HUVEC secreted ECM or purified individual ECM proteins as described previously¹³. Briefly, HUVEC ECM was obtained by culturing HUVECs in the wells to confluence and then detaching them with a solution of 5 mM EDTA in PBS. Individual ECM proteins were coated by overnight incubation at 10 μ g/ml in PBS. Collagen IV was obtained from Sigma. Laminin was obtained from Becton Dickinson (Bedford, MA). Prior to use, coated wells were washed two times with PBS.

Cell Adhesion Assay

To assess the effects of the above function-blocking anti-integrin mAbs on the ability of Calu-1 cells to establish adhesion, the following procedure was carried out, which is a modification of a previously published method⁹. Calu-1 cells were labeled with the radioactive ⁵¹Cr by adding 350 mCi of Na₂CrO₄ to the culture medium in a confluent T25 flask and incubating overnight. The labeled cells were detached from the flask with 0.25% Trypsin/5 mM EDTA in PBS and rinsed three times in DMEM with 1% BSA. Then, the blocking antibodies were mixed in with the cell suspension and 3×10^4 of ⁵¹Cr-labeled cells were added to prepared wells of a 48 well plate (that had been pre-incubated with DMEM containing 1% BSA for 30–60 min at 37°C), and incubated at 37°C for 2 h. After incubation, the non-adherent cells were removed by gently washing the wells three times with plain DMEM. The adherent cells were lysed with 1% SDS and removed to scintillation tubes with cotton swabs. The radioactivity of each sample collected was measured in a GammaTrac 1191 gamma counter, (Tm Analytic, Elk Grove Village, IL), and compared to standards comprised of reserved cell suspensions identical to the ones incubated in the wells. The assays were performed in triplicate.

Motility Assay

Assessment of planary cell motility was done using the computer-controlled motorized stage, environmental chamber, and video microscopy system described previously¹³. This system allows us to simultaneously obtain time lapse sequences of cell migration behavior in separate wells of a multiwell plate over a six-hour time period. The images of migrating cells were recorded every 10 min for 6 h, and tracks of the cell centroids were analyzed using the persistent random walk model, which mathematically quantifies the behavior of migrating cells^{1, 2}:

$$\langle d^2(t) \rangle = 4\mu P \left[\frac{t}{P} - (1 - e^{-t/P}) \right], \quad (1)$$

where μ is a random motility coefficient, analogous to a molecular diffusion coefficient and P is the directional persistence time, a statistical measure of the time scale in which a cell migrates without making a significant direction change. The model fitting and statistical analysis procedures used have been fully described¹³.

The fraction of cells which were motile in each condition was also calculated. A cell was considered to be motile if the root mean squared distance ($\sqrt{\langle d^2(t) \rangle}$) traveled over 100 min was greater than 10 μ m, and the fraction of motile cells, ϕ , is simply the number of motile cells divided by the total number of cells analyzed.

To assess the dependence of Calu-1 cell motility on integrin receptors, function-blocking mAbs directed against various α integrin subunits were added separately or in combination to cells at a final concentration of 10 μ g/ml for each mAb before placing them in the multiwell plates. Cells were allowed to adhere for 1 h in the wells in the presence of mAbs before the fields of view were chosen for observation of cell motility. Because the anti- $\beta 1$ mAb abolished Calu-1

cell adhesion to laminin when used at a sufficient concentration, a sub-saturating concentration of 1:625 final dilution of ascites was added after cells were allowed to adhere for 1 h, which reduced adhesion to about 25% of control (i.e., no antibody).

Equation 1 was fit to the cell track data using KaleidaGraph™ (Synergy Software, Reading, PA), which uses the Levenberg-Marquandt algorithm of chi-squares error minimization to compute the model parameters as well as the s.e.m. of each. A generalized nonlinear mixed-effect modeling procedure, MIXNLIN (MIXed-effects NonLINear Regression) with the SAS® program (SAS Institute, Cary, N.C.)¹², was used to statistically compare the model parameters quantifying the cell behaviors under different conditions. This method performs both nonlinear curve fitting and analysis of variance (ANOVA) to determine if there are statistically significant differences between both the response variable – $\langle d^2(t) \rangle$ – and the fitted model parameters (μ and P) under different conditions.

Results

Effects of anti-integrin function-blocking mAbs on Calu-1 adhesion

Because cell adhesion to and migration on extracellular matrix are generally believed to be mediated by integrins, we used blocking mAbs against $\alpha 2$, $\alpha 3$, $\alpha 6$, and $\beta 1$ subunits to identify on which specific integrin(s) these behaviors depend. These integrin subunits were selected because they were expressed at significantly higher levels on the malignant Calu-1 cells than on the non-malignant L132 cells and because $\alpha 2\beta 1$, $\alpha 3\beta 1$, and $\alpha 6\beta 1$ are receptors for collagen, laminin and fibronectin, ECM proteins on which the motility of Calu-1, but not L132, cells was significantly increased¹³.

In general, these anti- α subunit mAbs had little or no effect on the adhesion of Calu-1 cells to any of the substrates tested with the present method. As shown in Figure 1, individual anti- $\alpha 2$, - $\alpha 3$, and - $\alpha 6$ mAbs alone or in combination only minimally affected Calu-1 cell adhesion to laminin (Figure 1A), collagen IV (Figure 1B), HUVEC ECM (Figure 1C), and HUVEC monolayers (Figure 1C) even at saturating concentrations. In contrast, anti- $\beta 1$ mAb had a significant inhibitory effect on Calu-1 cell adhesion to laminin (Figure 2), even at sub-saturating dilutions as determined by FACS analysis.

Effects of integrin functional blocking mAbs on Calu-1 motility

The effects of anti- $\alpha 2$, - $\alpha 3$, - $\alpha 6$, and - $\beta 1$ mAbs on the Calu-1 cell motility on laminin and collagen IV were studied for two reasons. First, comparison of Calu-1 and L132 cell motility on individual ECM proteins showed that the former cells had significantly higher motility than the latter cells on both substrates¹³. Second, laminin and collagen IV are among the most abundant proteins present in the ECM of endothelial cells, with the former being the primary component of the basal lamina, and the latter creating the basic structure to which other proteins are bound in the layer below the basal lamina, the lamina densa⁷. After determining which mAbs have the most significant inhibitory effect on Calu-1 cell motility on these ECM components, we then tested the effects of these mAbs on Calu-1 motility on HUVEC ECM and on the HUVECs themselves.

Blocking mAbs Abolish Motility on Laminin—The effects of the blocking mAbs on Calu-1 migration on laminin were quantified by the best-fit motility parameters μ (Figure 3A) and ϕ (Figure 3B). When applied individually, the blocking mAbs to the three α subunits significantly reduced μ to about 80% of control in two of the three cases ($p = 0.082$, 0.0006 , and 0.051 for $\alpha 2$, $\alpha 6$, and $\alpha 3$, respectively). When applied in pair-wise combination, further reductions in motility were seen: anti- $\alpha 3$ and - $\alpha 6$, or anti- $\alpha 2$ and - $\alpha 6$ reduced μ to about half of the control value ($p = 0.0001$ for both cases), whereas anti- $\alpha 2$ and - $\alpha 3$ reduced μ to about

80% of control ($p = 0.051$). However, the motile fraction ϕ was not changed in any of these cases. Combining the mAbs against all three α subunits – $\alpha 2$, $\alpha 3$, and $\alpha 6$ – reduced μ to about 5% of control. Motility was also completely abolished by the anti- $\beta 1$ mAb. Although p -values could not be computed in these latter two cases (the matrices used in the calculations became ill-conditioned), statistical analysis was not needed, as the severe reduction in motility was so clearly evident. Interestingly, these were also the only two cases in which the motile fraction, ϕ , was affected: about 50 and 80% of the cells were immotile in the presence of all three anti- $\alpha 2$, - $\alpha 3$, and - $\alpha 6$ mAbs and of the anti- $\beta 1$ mAb, respectively. Note that μ and ϕ measure different aspect of cell motility. When μ is reduced while ϕ remains the same, the cells have reduced motility but keep their motile phenotype. By comparison, a reduction in ϕ indicates loss of the motile phenotype in some of the cells, which usually occurs concurrently with a reduction in the μ value.

The assay used here allows us not only to quantify the cells' motility using the parameters μ and ϕ , but also to observe morphological changes in response to the blocking mAbs. These are shown as 'stacked' images of the cells migrating on laminin in the absence (Figure 4A) and presence of anti- $\alpha 2$, - $\alpha 3$, and - $\alpha 6$ (Figure 4B) and anti- $\beta 1$ (Figure 4C) mAbs. As can be seen, in the absence of blocking mAbs, Calu-1 cells exhibited both high dispersion in their trajectories and an elongated shape typical of migrating cells. By contrast, in the presence of the blocking mAbs, very little dispersion was seen. These differences in dispersion correlate well with the calculated values of μ and ϕ discussed above. In addition, a clear difference can be seen between the two blocking cases that is not evident from the motility parameters – while there was little or no migration in both cases, the morphology of the cells was quite different. In the presence of the anti- $\beta 1$ mAb, the cells remained quite round throughout the observation period. In the presence of the anti- $\alpha 2$, - $\alpha 3$, and - $\alpha 6$ mAbs, by comparison, even though there is little dispersion, most of the cells were able to achieve an elongated morphology. This is consistent with the data of the adhesion assay where the anti- $\beta 1$ mAb is more effective in blocking adhesion than the anti- $\alpha 2$, - $\alpha 3$, and - $\alpha 6$ mAbs combined (Figures 1 and 2).

These results indicate that the migration of the Calu-1 cells on laminin requires at least one of the three integrins: $\alpha 2\beta 1$, $\alpha 3\beta 1$, and $\alpha 6\beta 1$, and that these integrins are used by the cells in a redundant fashion.

Blocking mAbs Reduce Motility on Collagen IV—Figure 5 shows the motility parameters for the Calu-1 cells migrating on collagen IV in the continuous presence of the anti- $\alpha 2$ and - $\alpha 3$ mAbs (both alone and in combination), as well as the anti- $\beta 1$ mAb. Blocking antibody against the $\alpha 6$ integrin subunit was not tested in this case, as it is not believed to bind to collagen IV⁶. Calu-1 motility on collagen IV was found to depend partly on the α integrin subunits, as μ (Figure 5A) was reduced to about half of the control case by mAbs against $\alpha 2$, or $\alpha 3$, or a combination of the two ($p = 0.0001$ for all three cases). As was true in the laminin case, the anti- $\beta 1$ mAb drastically reduced Calu-1 motility on collagen IV, with a significant and large reduction in μ : to about 20% of control ($p = 0.0001$). None of the antibodies tested had a large effect on ϕ , with 80% or more of the cells being motile in all cases tested (Figure 5B).

Figure 6 shows the 'stacked' representation of the Calu-1 cells migrating on collagen IV in the absence (A) and presence of the anti- $\alpha 2$ and - $\alpha 3$ (B) and the anti- $\beta 1$ (C) mAbs. Similar to what was previously seen on laminin, in the absence of blocking mAbs, the Calu-1 cells migrated on collagen IV with highly dispersed trajectories and an elongated shape. In the presence of the blocking antibodies, in comparison, the dispersion was markedly reduced, corresponding to the reductions in μ seen in Figure 5A. Unlike on laminin, however, the cells spread and achieved an elongated morphology on collagen IV, even with the blocking antibodies present.

Blocking Antibodies Reduce Motility on HUVEC ECM and HUVEC Monolayers

—The motility of Calu-1 cells on HUVEC ECM and HUVEC monolayers was assayed in the absence and presence of either a combination of the anti- $\alpha 2$, - $\alpha 3$, and - $\alpha 6$ mAbs or the anti- $\beta 1$ mAb. As reflected by the parameter μ (Figure 7A), the Calu-1 motility on both the HUVEC ECM and the HUVECs themselves did depend on these integrin subunits. On the HUVEC ECM, the reduction in μ from the control level was small but statistically significant when the combination of $\alpha 2$, $\alpha 3$, and $\alpha 6$ blocking mAbs were present ($p = 0.0001$). When the $\beta 1$ blocking mAb was present, μ was reduced to about one-third of the control level ($p = 0.0001$). In comparison, on the HUVEC monolayer, μ was reduced to about one-half of the control level by both the combination of the $\alpha 2$, $\alpha 3$, and $\alpha 6$ blocking mAbs and by the anti- $\beta 1$ mAb ($p = 0.0001$ for both cases). As was true in the collagen IV cases, these reductions in motility parameters were not accompanied by a reduction in the ϕ , with 80% or more of the cells remaining motile in the presence of the blocking antibodies (Figure 7B).

The morphology of the Calu-1 cells migrating on HUVEC ECM and on monolayers of HUVECs is shown in Figures 8 and 9, respectively. When no blocking mAbs were present, the Calu-1 cells had significantly dispersed trajectories and typically elongated morphology, including some cells migrated long distances on both substrates (Figures 8A and 9A). The combination of anti- $\alpha 2$, - $\alpha 3$, and - $\alpha 6$ mAbs did not affect the dispersion of Calu-1 cell trajectories on HUVEC ECM (Figure 8B) to the large degree that it did on laminin (Figure 4B), but the anti- $\beta 1$ mAb did (Figures 8C and 4C). Again, the reduced amount of trajectory dispersion was well reflected in the reduced μ values (Figure 7A). In the presence of all three anti- $\alpha 2$, - $\alpha 3$, and - $\alpha 6$ mAbs or the anti- $\beta 1$ mAb, many of the cells were seen to become more rounded (Figure 8, B and C).

On the HUVEC monolayers, the migration and morphology of the cells in the presence of the anti- $\alpha 2$, - $\alpha 3$, and - $\alpha 6$ mAbs (Figure 9B) were quite similar to those with the anti- $\beta 1$ mAb (Figure 9C). These panels also highlight a phenomenon which was often seen: although the majority of the cells may show decreased motility, a few were seen to migrate significantly. This is not an entirely unexpected result, as the Calu-1 cells are heterogeneous in nature.

Discussion

Tumor cell motility is an integral element of the metastasis process. In blood-borne metastasis, it is required for the cells to invade the endothelial and basement membrane layers at distant sites in the vasculature. As such, migration of malignant cells may represent a target of intervention against the metastatic processes. We seek in this study to expand the understanding of the cellular processes employed in extravasation through blood vessel walls.

Our hypothesis is that tumor cells, via increased expression of cell surface receptors, are capable of opportunistic use of molecules secreted by the endothelial cells themselves to achieve the required motility. In a recent study, we showed an increase in migratory behavior of the malignant Calu-1 lung epidermoid carcinoma cell line over the non-malignant L132 lung epithelial cell line¹³. The motility of Calu-1 cells, but not L132 cells, was upregulated by HUVEC monolayers, HUVEC ECM, as well as purified ECM proteins collagen I, collagen IV, fibronectin and laminin. Concomitant expression levels of the $\alpha 2$, $\alpha 3$, $\alpha 6$, and $\beta 1$ integrin subunits (which are known to bind to extracellular matrix proteins) were found to be significantly higher on the Calu-1 cells than on the L132 cells. Other ECM binding integrins were expressed either at significant but comparable levels ($\alpha 5$ and αv subunits) or low levels on both cell lines ($\alpha 1$, $\alpha 4$, $\beta 3$, and $\beta 4$ integrin subunits).

It is known that ligands for the $\alpha 2\beta 1$ integrin include collagen and laminin, ligands for the $\alpha 3\beta 1$ integrin include fibronectin, laminin and collagen, and the ligand for the $\alpha 6\beta 1$ integrin

is laminin. Thus, the previous data established a correlation between integrin expression and motility – the Calu-1 cells, with their higher levels of expression for ECM binding integrins, were more motile than the L132 cells on these ECM proteins. In order to go beyond this correlative observation, in this work we investigated the dependence on the integrins identified of the Calu-1 cell adhesion to and migration on the target substrates by adhesion and motility measurements in the presence and absence of function-blocking antibodies. Motility was measured in three ways – motility coefficient μ , motile fraction ϕ , and the stacked images – to obtain complementary information.

Because Calu-1 cells express multiple integrin receptors for the particular purified ECM proteins studied, function-blocking mAbs against different integrin subunits were used separately and in combination in order to assess the relative contributions from each integrin. Antibodies against the $\alpha 2$, $\alpha 3$, and $\alpha 6$ integrin subunits, used both alone and in combination, as well as a mAb against the $\beta 1$ integrin subunit significantly reduced Calu-1 migration on laminin, collagen IV, HUVEC ECM, and HUVEC monolayers (Figures 3, 5, and 7), identifying the migratory supportive role of $\alpha 2\beta 1$, $\alpha 3\beta 1$, and/or $\alpha 6\beta 1$ integrins. All three integrins bind laminin; however, their contributions to Calu-1 cell motility on laminin are not additive. Blocking all three α chains simultaneously caused a greater inhibition of Calu-1 cell motility on laminin than the sum of the inhibitory effects of each individual anti- α antibody (Figure 3). This suggests that when one of the integrins was blocked, the other two integrins were able to compensate partly for the lost function. In other words, these integrins may have overlapping and redundant function in supporting migration on laminin. In comparison, Calu-1 cells express two receptors for collagen IV: $\alpha 2\beta 1$ and $\alpha 3\beta 1$ integrins. Their contributions to Calu-1 cell motility on collagen IV are also not additive but in a way different from those seen in the laminin case. Blocking both α chains simultaneously caused a total reduction in motility that was no more than the reduction caused by blocking either α chain alone (Figure 5). This suggests that the two integrins are both required for the Calu-1 cells to migrate on collagen IV.

Interestingly, the anti- $\beta 1$ mAb exhibited a stronger inhibitory effect on migration than the combined effects of the three anti- α subunit mAbs in all cases tested except for migration on HUVEC monolayers in which the anti- $\beta 1$ mAb had the same level of inhibition as the three anti- α subunit mAbs (Figures 3, 5, and 7). In addition, the dependence of Calu-1 adhesion and migration on the integrins tested was not necessarily correlated. Both adhesion to and migration on laminin were dependent on $\beta 1$ integrins, as the function-blocking antibody against $\beta 1$ severely inhibited both (Figures 2 and 3). However, the anti- $\beta 1$ blocking antibody caused substantial reductions in Calu-1 cell motility on collagen IV, HUVEC ECM, and HUVEC monolayers without causing significant reductions in Calu-1 cell adhesion to any of these substrates (Figures 5 and 7 and data not shown). Likewise, the combination of blocking antibodies against $\alpha 2$, $\alpha 3$, and $\alpha 6$ integrin subunits reduced motility on (Figures 3, 5, and 7) but not adhesion to any of laminin, collagen IV, HUVEC ECM, or HUVEC monolayers (Figure 1 and data not shown). In the motility assay, the cells were allowed to adhere for 1 h to the substrate prior to the addition of the anti- $\beta 1$ mAb, which was used at a sub-saturating concentration. The cells remained adherent in the presence of the anti- $\beta 1$ mAb. However, they had a very round morphology throughout the six hour observation period, suggesting that they were unable to form new attachments and spread (Figure 4C). Since the cells were able to remain adherent, it appears that the antibody did not diffuse into the region where the cells had already established adhesion to the substrate and/or compete for ligand in that region. In comparison, the combination of blocking antibodies against the $\alpha 2$, $\alpha 3$, and $\alpha 6$ integrin chains substantially inhibited motility on laminin without reducing adhesion (Figure 1) and with much less reduction in spreading (Figure 4B). This suggests that motility depends on more than just the ability to form new attachments between the cell and the substrate. In this case, the cells were able to spread via mechanisms other than those which were blocked (which allowed adherence but did not trigger motility).

The separated roles of integrins in adhesion and motility have also been found in other studies. For example, the human colon carcinoma cell line LIM1215 has been reported to require $\alpha 2\beta 1$ and $\alpha 3\beta 1$ integrins for adhesion to laminin-10 under unstimulated conditions, while motility was mediated by $\alpha 3\beta 1$ (but not $\alpha 2\beta 1$ or $\alpha 6\beta 4$)¹⁰. Also, mouse mammary carcinoma cells were reported to require both $\alpha 6$ and $\beta 1$ integrins to migrate through laminin coated filters, but to require $\beta 1$ (and also some $\alpha 1$) integrins to adhere to the laminin⁵. In the latter example, the difference in roles among integrins was linked to regulation of stromelysin-1 expression by $\alpha 1$ and $\alpha 2$ integrins, but not by $\alpha 6$ or $\beta 1$ integrins⁵. Another study reported that blocking the function of the $\alpha 3$ integrin subunit inhibited migration of malignant mesothelioma cells to laminin and collagen IV without affecting adhesion, while other integrin mAbs inhibited both adhesive and motile behaviors³.

One possible explanation for the separation of functionality between integrins used by the cells for adhesion and motility may be that the adhesion and migration processes are controlled by separate mechanisms. Alternatively, the cells could be adhering to a different ECM protein(s), such as fibronectin, which could come from the serum used in the media or could be secreted from the Calu-1 cells themselves. The cells could bind to fibronectin via the $\alpha 5\beta 1$ integrin (which is a fibronectin receptor only¹¹), which was shown previously to be expressed at significant levels on both Calu-1 and L132 cells. An argument against this explanation is that the cells did not adhere or migrate well on untreated tissue culture plastic, vitronectin, or gelatin, and presumably the cells could have bound to fibronectin in these cases as well. Other possible explanations include: adhesion and migration via activation of different signaling pathways by different integrins, via spatial regulation of integrin affinity, and by the use of additional receptors, such as the 67 kDa laminin receptor. Several signaling pathways have been linked to integrins, including via the Ras superfamily of GTPases (such as the Ras/Raf/MEK/MAPK pathway) as well as the Rho-family (including Rho, Rac, and CDC42) and the Src-family kinases. These pathways can affect an integrin's affinity for its ligands and play a part in the cytoskeletal reorganization that occurs in cell motility⁸. Future studies are required to elucidate these signaling pathways.

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Figure 1.
Lack of Inhibitory Effect of Anti- α Subunit Blocking mAbs (10 μ g/ml) on Calu-1 Adhesion to Laminin.

Figure 2. Calu-1 Cell Adhesion to Laminin Was Blocked by Anti-β1 mAb in a Dose-Dependent Fashion. Calu-1 cell adhesion to laminin coated tissue culture plastic after two hours of incubation was measured in the continuous presence of the anti-β1 mAb. The adherent fraction decreases with increasing mAb concentration, showing a strong inhibitory effect even at sub-saturating concentrations.

Figure 3.

Effect of Blocking mAbs on Motility on Laminin. Motility coefficient (A) and motile fraction (B) calculated for Calu-1 cells migrating on laminin coated tissue culture plastic in the presence of blocking mAbs against the indicated integrin subunits. Because these were studied in a series of experiments (indicated by different fill patterns), motility on laminin with no mAbs present (None) for each experiment is plotted alongside the test conditions to provide a positive control. * = statistically significant difference from the respective control; # = motility too low to allow statistical analysis. Error bars = s.e.m.

Figure 4.

Morphology of Migrating Cells on Laminin in the Absence (A) and Presence of Anti- $\alpha 2$, - $\alpha 3$, and $\alpha 6$ (B) and Anti- $\beta 1$ (C) mAbs. Planar projections of cells in one field of view of each condition (six fields of view per condition were analyzed to calculate the motility parameters) were recorded once every 10 minutes and stacked upon each other. Bar = 50 μm .

Figure 5.

Effect of Blocking mAbs on Motility on Collagen IV. Motility coefficient (A) and motile fraction (B) calculated for Calu-1 cells migrating on collagen IV coated tissue culture plastic in the presence of blocking mAbs against the indicated integrin subunits. * = statistically significant difference from control (no antibody). Error bars = s.e.m.

Figure 6. Morphology of Migrating Calu-1 Cells on Collagen IV in the Absence (A) and Presence of Anti- $\alpha 2$ and - $\alpha 3$ (B) and Anti- $\beta 1$ (C) mAbs. Bar = 50 μm .

Figure 7.

Effect of Blocking mAbs on Motility on ECM and HUVEC Monolayers. Motility coefficient (A) and motile fraction (B) calculated for Calu-1 cells migrating on HUVEC ECM (filled) and on HUVEC monolayers (open) in the presence of blocking mAbs against the indicated integrin subunits. * = statistically significant difference from the respective control (no antibody). Error bars = s.e.m.

Figure 8. Morphology of Migrating Calu-1 Cells on HUVEC ECM in the Absence (A) and Presence of the Anti- $\alpha 2$, - $\alpha 3$, and - $\alpha 6$ (B) and the Anti- $\beta 1$ (C) mAbs. Bar = 50 μm .

Figure 9. Morphology of Migrating Calu-1 Cells on HUVEC Monolayers in the Absence (A) and Presence of the Anti- α 2, - α 3, and - α 6 (B) the Anti- β 1 (C) mAbs. Bar = 50 μ m