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The L1 Adhesion Molecule Supports $\alpha v\beta 3$-Mediated Migration of Human Tumor Cells and Activated T Lymphocytes

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The L1 adhesion molecule is a member of the immunoglobulin superfamily which is expressed by neural and hematopoietic cells. L1 is primarily a cell surface molecule but in its released form it becomes embedded in the extracellular matrix. In addition to the established L1-L1 homotypic interaction, L1 can bind to $\alpha v\beta 3$ in the human. The 6th Ig-like domain is critical for this function. We now demonstrate that a fusion protein containing the 6th Ig-like domain of L1 (6.L1-Fc) can support the migration of human MED-B1 ($\alpha v\beta 3^+$) but not of Nalm-6 cells ($\alpha 5\beta 1^+$). The migration was blocked in the presence of a mab to $\alpha v\beta 3$ and was not seen on a 6.L1-Fc in which the RGD site was mutated. Activation of human T lymphocytes in the presence of PHA and PMA led to the induction of $\alpha v\beta 3$ and $\alpha v\beta 5$ expression and concomitantly induced migration of the cells on 6.L1-Fc. The migration was blocked by mabs to $\alpha v\beta 3$ but not to $\alpha v\beta 5$. Our results suggest that L1 exposed at the cell surface or as a matrix constituent can serve as a potent substrate for $\alpha v\beta 3$ mediated cell migration.

Cell adhesion and migration are initiated in many instances by the specific ligation of cell surface integrins. Integrins are heterodimeric cell adhesion molecules that were initially found to mediate the interaction of cells to components of the extracellular matrix like laminin, fibronectin, vitronectin etc. (1,2). In past years also cellular ligands to integrins were identified. Examples are the $\alpha 4$-integrins which bind to the vascular cell adhesion molecule VCAM-1 and the addressin MAdCAM-1 or the CD31 molecule that can bind to the $\alpha v\beta 3$ integrin (for review see 3). We have recently identified the L1 adhesion molecule as a cellular ligand for the fibronectin receptor $\alpha 5\beta 1$ in the mouse and the $\alpha v\beta 3$ integrin in the human (4,5).

L1 is a 200-240 kDa transmembrane glycoprotein which consists of 6 Ig-like domains and five fibronectin-type III repeats (6-8). L1 was originally recognized as a neural adhesion molecule shown to be involved in granule neuron migration in the developing mouse cerebellar cortex (9), the fasciculation of neurites (10) and neurite outgrowth on other neurites and Schwann cells (11,12). L1 expression was also found on hematopoietic cells in mouse and humans (5,13). On mouse leukocytes L1 was found to play a role in the binding to endothelial cells (14). In addition to its function as a cell surface adhesion molecule L1 can be shed from the cell surface (15) and be deposited in the ECM (15,16) which suggests a potential role for L1 as a matrix constituent.

Evidence exists that $\alpha v\beta 3$ ligation can promote cell motility or migration (17). Given the finding that human L1 is a novel ligand to this integrin we now addressed the question whether this interaction can promote cell migration.

MATERIALS AND METHODS

Cells and antibodies. The mabs used in this study as well as the isolation of human blood mononuclear cells has been described (5). T cells (3×10^6cells/ml) were activated in RPMI 1640 supplemented with 10% FBS and 1 μg/ml PHA with or without 100ng/ml PMA. On day 3 of culture, the blast fraction was harvested using Percoll gradient centrifugation as described (18). Human tumor cell lines Nalm-6 and MED-B1 have been described (5).

Cytofluorography. Cytofluorographic analysis of cells with monoclonal antibodies has been described (5).

Construction of Ig chimeric fusion protein. The construction of human 6.L1-Fc has been described in detail before (5). A mutant 6.L1-Fc without RGDs was obtained in the following way: a mouse 6.L1-Fc was mutated in both RGDs in such a way that R was changed to L. This results in a protein sequence which is nearly identical to the human 6.domain except for the substitutions R554L and R575K. The detailed production and characterisation of this mutant will

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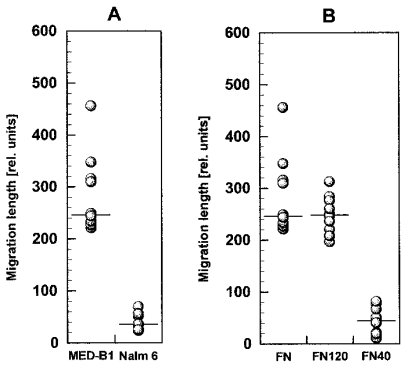

FIG. 1. Migration of MED-B1 and Nalm-6 cells on fibronectin and vitronectin. Cells were allowed to migrate for 2 hrs at 37°C under constant observation. A) Migration of MED-B1 cells and Nalm-6 cells on vitronectin. B) Cell migration of MED-B1 cells on fibronectin and subfragments FN120 and FN40. The cell diameter is appr. 20 rel. units. Each dot represents an analyzed cell.

be described elsewhere (S.Katich, S.Schöllhammer, O.Ebeling and P.Altevogt, in preparation). Plasmid-DNAs were transiently transfected into COS cells and Fc-fusion protein were purified as described (5). The human P-Selectin-Fc fusion protein was a gift from Genetics Institute, Boston, USA.

Cell migration assay. Migration experiments were performed in 16 well LABTEK glass chamber slides (Nunc, Wiesbaden, Germany). Substrates were coated at 4°C for overnight in PBS using predetermined concentrations. The slides were blocked for 30 min at 37°C with 1% ovalbumin in PBS and then washed with HBSS. 5×10^4 cells in 100 μl migration buffer (HBSS containing 0.5 mM Mn^{2+}) were seeded on the glass slide and incubated at 37°C on a temperature-controlled microscope stage. Cell migration was recorded with a video camera connected to an Apple computer using NIH Image software (version 1.56). Over a period of 2 hr a frame was taken every 60 seconds and stored on an optical disk. In all experiments frames with identical size and magnification of the microscope were used. The percentage of migratory cells varied between 20-25% for tumor cells and 30-50% for activated T lymphoblasts. For blocking of migration, antibodies were added at a concentration of 20 μg/ml to the cell suspension before seeding to the glass slides. Viability of the cells was controlled at the onset and the end of the experiment by trypan blue staining. The viability of the cells did not drop below 90 % during the assay. The NIH Image programme was used to quantify the migration length of a single cell between two time points. Cell migration is given in relative units. The cell diameter for tumor cells is appr. 20 relative units the diameter for activated T lymphoblasts appr. 10 relative units.

RESULTS

Migration of Tumor Cells

The human tumor cells MED-B1 express $\alpha v\beta3$ and are negative for $\alpha v\beta5$ and the fibronectin receptor $\alpha5\beta1$ as shown by immunoprecipitation and FACS analysis. Conversely, Nalm-6 tumor cells only express $\alpha5\beta1$ and no αv-integrins (data not shown and (5)). We investigated whether contact of MED-B1 or Nalm-6 cells with substrate-bound vitronectin or fibronectin could induce cell migration. The migratory behavior of the cells was recorded by video microscopy for 2 hrs at 37°C. Fig. 1A shows a representative migration experiment of MED-B1 and Nalm-6 cells on vitronectin coated at 10 μg/ml. It is evident that within the assay duration the MED-B1 cells showed migration of up to 20 fold of their diameter in distance. The migration was non-directed. MED-B1 cells also migrated on fibronectin and the 120 kDa subfragment of fibronectin (FN120) containing the RGD site but not on the FN40 subfragment (Fig.1B). Nalm-6 cells showed adhesion to fibronectin (5) but were unable to migrate on this substrate even at higher coating concentrations. Nalm-6 cells did also not migrate on vitronectin (Fig.1A).

A fusion protein containing the Fc portion of human IgG1 and the 6.domain of human L1(6.L1-Fc) supports adhesion of MED-B1 (5). We studied whether the recombinant protein also promotes migration of the tumor cells. As shown in Fig. 2A the MED-B1 cells migrated well on 6.L1-Fc and this migration was completely blocked in the presence of the $\alpha v\beta3$ specific mab but not by an isotype-matched control mab. Nalm-6 cells did not migrate on the 6.L1-Fc protein consistant with our previous finding that the RGD sequence in 6.L1-Fc is not recognized by the human $\alpha5\beta1$ integrin (5).

To study the importance of the RGD sites in the 6. domain of L1 for MED-B1 migration and to exclude the possibility that the Fc portion of the fusion protein obscured the results we carried out control experiments. As shown in Fig. 2B a human P-Selectin-Fc fusion protein did not promote migration of MED-B1 cells although the cells were able to bind to the immobilized protein (not shown). We also used a 6.L1-Fc protein in which the R in position 554 and 575 had been changed (R554L respectively R575K) so that no RGD and inverse RGD sites are present. As shown in Fig.2B no migration of MED-B1 was observed on the mutant protein.

Induction of αv Integrins on Human T Lymphocytes

We adressed the ability of human peripheral T lymphocytes to use the 6.L1-Fc as substrate for cell migra-

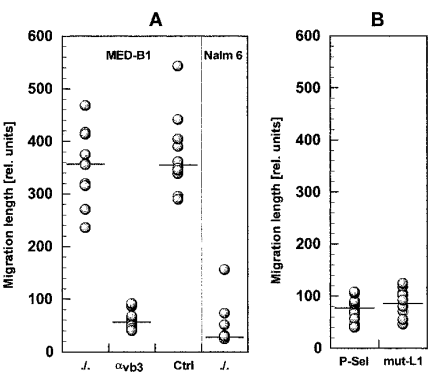

FIG. 2. Migration of MED-B1 cells on a 6.domain L1-Fc fusion protein. (A) Migration of MED-B1 on the 6.L1-Fc and blocking by mab to the $\alpha v\beta3$ integrin. (B) Lack of migration of MED-B1 cells on human P-selectin-Fc and a 6.L1-Fc without RGD (mut L1-Fc).

tion. Preliminary experiment with resting T lymphocytes indicated that the cells were immobile on this substrate and did not express αv integrins (data not shown). T lymphocytes were therefore activated with PHA or with a combination of PHA and PMA for 3 days (19) and then analysed for αv expression. As shown in Fig. 3A only the activation with PHA and PMA led to an appreciable induction αvβ5 and αvβ3 integrin expression.

Migration of T Lymphoblasts on Human 6.L1-Fc

Activated T lymphoblasts were analysed for their ability to migrate on 6.L1-Fc fusion protein. As shown in Fig.3B the PHA activated cells showed little migration comparable to the behavior of Nalm-6 tumor cells. In contrast, the PHA/PMA activated cells migrated significantly better on 6.L1-Fc and this migration was completely blocked in the presence of the αvβ3 specific mab. No inhibition was observed in the presence of the αvβ5 specific mab indicating that the migration of activated T lymphocytes was solely supported by the αvβ3 integrin.

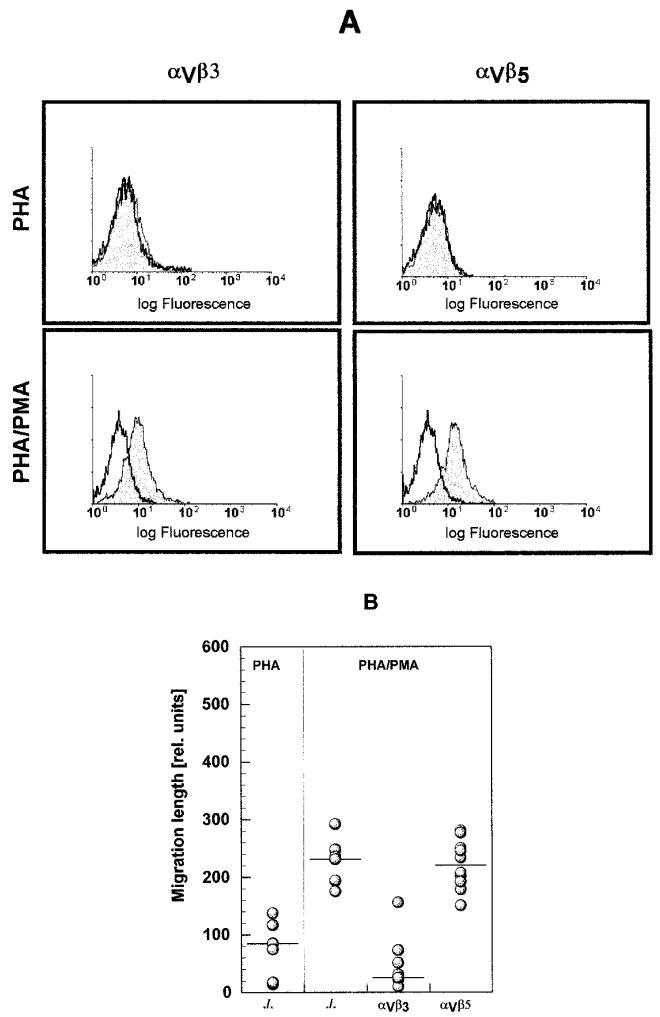

FIG. 3. Induction of av integrin expression on human T lymphoblasts and migration on 6.L1-Fc. (A) Human lymphocytes were activated by PHA or PHA/PMA and the integrin profile was measured by FACS analysis using indirect immunofluorescence. Filled curves represent staining with the indicated mabs. Open curves are background controls. (B) Migration of T lymphoblasts on 6.L1-Fc is blocked by mabs to the αvβ3 integrin.The cell diameter is appr. 10 rel. units.

DISCUSSION

We have analyzed the ability of human L1 to support the αvβ3 induced migration of cells. Since L1 has several cellular binding sites we used a fusion protein derived from the 6.domain which was shown to be critical for integrin-mediated cell adhesion (5,15). We observed migration of human tumor cells and activated T lymphocytes on the L1 substrate which could be blocked by αvβ3 specific antibodies and which was RGD dependent. Our results confirm and extend earlier data of Montgomery et al. who showed that complete human and rat L1 can support αvβ3 induced haptotactic cell migration (15).

Under physiologic conditions L1 could serve as a substrate for cell migration when bound to a cell surface as well as when deposited in the extracellular matrix. L1 has structural and functional similarities with CD31, another member of the Ig superfamily. Both molecules are composed of 6 Ig-like and can bind in a homotypic as well as in a heterotypic fashion (20,21). Both CD31 (21) and L1 (5,15) have been identified as cellular ligands for αvβ3 integrin in the human and are expressed by many different cell types including leukocytes and endothelial cells (5, and unpublished results). Mabs to CD31 can block adhesion and transmigration of leukocytes through monolayers of endothelial cells (22,23). In previous work we demonstrated that L1 was also involved in the binding to endothelial cells (14). It is possible that the adhesion pairs αvβ3-CD31 and αvβ3-L1 play a major role in the arrest and initiation of cell migration of leukocytes on endothelial cells which preceeds transmigration (3).

Several types of cells including neurones, leukocytes and melanoma cells release L1 from the cells surface under appropriate conditions (see 24). It is not known whether shedding from the cell surface is necessary for the deposition of L1 into ECM.

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