Original Article

Blockade of β1 Integrin–Laminin-5 Interaction Affects Spreading and Insulin Secretion of Rat β-Cells Attached on Extracellular Matrix

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When attached on a matrix produced by a rat bladder carcinoma cell line (804G matrix), rat pancreatic β-cells spread in response to glucose and secrete more insulin compared with cells attached on poly-L-lysine. The aim of this study was to determine whether laminin-5 and its corresponding cell receptor β1 integrin are implicated in these phenomena. By using specific blocking antibodies, we demonstrated that laminin-5 is the component present in 804G matrix responsible for the effect of 804G matrix on β-cell function and spreading. When expression of two well-known laminin-5 ligands, β1 and β4 integrin, was assessed by Western blot and RT-PCR, only the β1 integrin was detected in β-cells. Anti–β1 integrin antibody reduced the spreading of β-cells on 804G matrix. Blockade of the interaction between β1 integrins and laminin-5 resulted in a reduction in glucose-stimulated insulin secretion. Blocking anti–β1 integrin antibody also inhibited focal adhesion kinase phosphorylation induced by 804G matrix. In conclusion, anti–β1 integrin and –laminin-5 antibodies interfere with spreading of β-cells, resulting in decreased insulin secretion in response to glucose. Our findings indicate that outside-in signaling via engagement of β1 integrins by laminin-5 is an important component of normal β-cell function. *Diabetes* 55:1413–1420, 2006

The extracellular matrix plays a critical role in modulating epithelial cell morphology, growth, migration, and differentiation. The response of cells to extracellular matrix attachment is mediated primarily by the integrin family of adhesion receptors. Integrins are heterodimeric integral membrane glycoproteins composed of an α-subunit that is noncovalently linked to a β subunit (1,2). β1 integrins are expressed on most epithelial cells and bind to collagen, fibronectin, laminin-1 (3), and laminin-5 (4). These molecules have been suggested to play important roles in morphogenesis (5,6), cell differentiation (7), polarity, proliferation (8), and survival (8,9).

In the pancreas, laminin is the major component (80%) of the basement membranes that form the interface between epithelia and connective tissues (10,11), whereas fibronectin is mainly observed underneath endothelial cells and epithelial ducts (12). Both laminin and fibronectin have been shown to affect differentiation and proliferation of β-cells (13–15).

The importance of cell–extracellular matrix interactions for optimally regulated insulin secretion is supported by a number of studies on β-cells cultured on various crude matrices or their purified extracts (15–21). We have shown that isolated rat islet β-cells cultured on the matrix produced by 804G cells (referred to as 804G matrix) secrete twofold more insulin in response to glucose compared with cells cultured on plastic or poly-L-lysine (22,23). This matrix, deposited by 804G rat bladder carcinoma cells, is rich in laminin-5 (24–26), a heterotrimer protein consisting of α3, β3, and γ2 subunits, and an essential component of several epithelial basement membranes (27,28). Our previous study suggests that the effects of 804G matrix on β-cells are mediated, at least in part, by laminin-5 (22).

Within islets, it has previously been shown that α3β1 and α6β1 integrins are expressed on rat pancreatic β-cells (22,29), and both are receptors for laminin. Furthermore, the level of expression of α6β1 integrin by β-cells correlates with the rate of insulin secretion in response to glucose (22).

The aim of the current study was to demonstrate that the interaction between laminin-5 and β-cell β1 integrins is involved in the effect of 804G matrix on cell spreading and insulin secretion.

RESEARCH DESIGN AND METHODS

The antibodies used were hamster anti-rat CD29 (β1 integrin chain, Ha2/5), control hamster IgM, and fluorescein isothiocyanate–conjugated mouse anti-hamster from Becton Dickinson Pharmingen (San Jose, CA); anti–γ2 chain of laminin-5, anti–β4 integrin, and anti–focal adhesion kinase (FAK) from Santa Cruz Biotechnology (Santa Cruz, CA); rabbit anti–phospho-FAK (Tyr-397) from Biosource International (Camarillo, CA); anti-fibronectin, control mouse, and rabbit IgG from Sigma (Buchs, Switzerland); anti–α3 chain of laminin-5 (CM6), a gift from of Dr. Vito Quaranta (the Scripps Research Institute, La Jolla, CA); monoclonal anti-actin from Chemicon International (Temecula, CA); and anti–mouse–horseradish peroxidase (HRP), anti–rabbit-HRP, and anti–goat-HRP from Amersham Pharmacia Biotech (Dubendorf, Switzerland).

Islet isolation and β-cell purification. Islets of Langerhans were isolated by collagenase digestion of pancreases from 180- to 200-g male Wistar rats (Janvier, Le Genest-St-Isle, France) followed by Ficoll purification, using a modification of the method of Sutton et al. (30). Islets were trypsinized and

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DMEM, Dulbecco's modified Eagle's medium; FAK, focal adhesion kinase; HRP, horseradish peroxidase; KRBH, Krebs-Ringer bicarbonate HEPES buffer.

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β-cells purified, using a fluorescence-activated cell sorter (FACStar-Plus; Becton Dickinson, Sunnyvale, CA), as previously described (31,32), to yield a population of >95% β-cells.

804G matrix. 804G cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, Invitrogen, Basel, Switzerland) containing 10% FCS and 5.6 mmol/l glucose (33). At confluence, cells were washed and maintained for 3 days in DMEM without FCS. Conditioned medium was centrifuged (180*g*, 5 min), filtered (0.22 μm), and frozen at −20°C, and hereafter it will be referred to as 804G matrix.

Coating of dishes and slides. Petri dishes (35 mm diameter) were coated with 100 μg/ml fibronectin, 75 μg/ml vitronectin, or 100 mg/ml laminin from EHS sarcoma cells (Sigma) in 20 mmol/l carbonate, pH 9.6, and incubated for 90 min at 37°C, and nonspecific binding sites were blocked with 0.5% BSA for 30 min at 37°C. Aliquots (60 μl) of 804G matrix or 100 μg/ml poly-L-lysine were layered at the center of Petri dishes. The glass microscope slides used as Cunningham's chambers (34) were coated with 300 μl of 804G matrix or 100 μg/ml poly-L-lysine. After coating for 18–20 h in a damp box at 37°C, Petri dishes and glass slides were washed 3 times with sterile H_2O and air dried. Where appropriate, dishes coated with poly-L-lysine or 804G matrix were incubated for 2 h at 37°C with either anti–laminin-5 (100 μg/ml) or anti-fibronectin antibody (50 μg/ml) in PBS, 0.5% BSA. Nonimmune mouse or rabbit IgG were used as controls. Dishes were washed 3 times with PBS and air-dried.

Culture and attachment of cells to Petri dishes. Sorted β-cells were washed twice with DMEM, 10% FCS, 11.2 mmol/l glucose, 1 mmol/l sodium pyruvate, 110 units/ml penicillin, 110 μg/ml streptomycin, and 50 μg/ml gentamicin (control medium). Aliquots of 3×10^5 cells were seeded in nonadherent 100-mm-diameter Petri dishes containing 9 ml control medium and incubated for 20 h at 37°C to allow full recovery from islet isolation or cell purification. The cells were then resuspended at 4×10^5 cells/ml in the control medium, and 50-μl droplets were plated on Petri dishes coated as described. Cells were analyzed 24 h later. To evaluate the effect of anti–β1 integrin blocking antibody, cells were resuspended at a density of 10^6 cells/ml in DMEM containing 11.2 mmol/l glucose and 10% FCS and preincubated for 1 h with 2 g/μl anti–β1 integrin blocking antibody (Ha2/5) or purified hamster IgM or vehicle. Then, cells were plated as droplets at the center of Petri dishes coated with 804G matrix or poly-L-lysine, incubated for 5 h at 37°C, fixed with 4% paraformaldehyde (20 min, room temperature), and stained with Evans blue (0.06% in PBS). Phase-contrast views of different fields were photographed, and the cell profile area was measured, using ScionImage software (Fredrick, MD).

Insulin secretion assay and insulin content. β-Cells pretreated with 2 μg/ml Ha2/5 antibody as described above were washed twice with Krebs-Ringer bicarbonate HEPES buffer (KRBH; 125 mmol/l NaCl, 4.74 mmol/l KCl, 1 mmol/l $CaCl_2$, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4$, 5 mmol/l $NaHCO_3$, 25 mmol/l HEPES, pH 7.4, and 0.1% BSA) containing 2.8 mmol/l glucose and plated on poly-L-lysine– or 804G matrix–coated dishes. Purified hamster IgM (2 μg/ml) or vehicle were added as controls. Cells were cultured in the continued presence of the antibody for 2 h. Cells attached on Petri dishes were washed 3 times with KRBH and preincubated 1 h at 37°C with KRBH containing 2.8 mmol/l glucose, followed by successive 1-h incubations at 37°C in KRBH with 2.8 or 16.7 mmol/l glucose. Insulin was extracted from cells with acid-ethanol, and insulin in buffers and extracts were measured by radioimmunoassay with rat insulin as the standard. Total insulin is the sum of cellular insulin and insulin secreted during the first and second incubation periods.

Reverse hemolytic plaque assay. Insulin secretion from single β-cells was assessed by reverse hemolytic plaque assay, as previously described (22). Briefly, β-cells, pretreated 1 h at 37°C in suspension with Ha2/5 antibody or vehicle, were diluted in KRBH containing 2.8 mmol/l glucose. Packed sheep erythrocytes (5% vol/vol; Behring Institute, Marburg, Germany) previously coated with protein A were then mixed with β-cells, and 60-μl aliquots of this preparation were inserted in Cunningham's chambers. After a 2-h incubation at 37°C in the presence or absence of 1 or 2 μg/ml Ha2/5 antibody, the chambers were rinsed with KRBH containing either 2.8 or 16.7 mmol/l glucose and then filled with the same buffer supplemented with heat-inactivated (45 min at 56°C) anti-insulin guinea pig serum (1:300 dilution) (35). After a 1-h incubation at 37°C, chambers were rinsed with KRBH containing 2.8 mmol/l glucose, filled with the same buffer containing guinea pig complement (1:40 dilution; Behring Institute), and incubated 1 h at 37°C. Chambers were then filled with 0.04% (wt/vol) trypan blue in KRBH, rinsed with KRBH, and filled with Bouin's fixative. After rinsing in PBS, slides were covered with PBS-glycerol (1:2 dilution, vol/vol) and screened by microscopy.

Immunofluorescence. Cells were preincubated for 1 h in suspension with 2 μg/ml Ha2/5 antibody or purified hamster IgM and then attached on poly-L-lysine– or 804G matrix–coated glass. After 2 h of culture, cells were washed with PBS–0.5% BSA and incubated for 1 h at 4°C with fluorescein isothiocyanate–conjugated mouse anti-hamster monoclonal antibody. Then, cells were fixed with 4% paraformaldehyde (20 min, room temperature), rinsed, and mounted under glass coverslips. The preparations were observed with an Axiocam fluorescence microscope.

Western blot. Cells were washed with ice-cold PBS with 1 mmol/l sodium vanadate and lysed in sample buffer (62 mmol/l Tris-HCl, pH 6.8, 2% SDS, 5% glycerol, and 1% 2-mercaptoethanol). Protein concentrations were determined with the amido black method (36), and equal amounts of total protein were loaded for SDS-PAGE. All samples, after separation on an SDS-PAGE gel, were electroblotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) for immunoblotting with the appropriate antibody. An enhanced chemiluminescence protein detection kit (Amersham Biosciences) and a Kodak image station were used for visualization of the bands.

Semiquantitative RT-PCR. Total RNA was extracted, using a QIAshredder and RNeasy mini kits (Qiagen, Basel, Switzerland) according to the manufacturer's instructions, and stored at −80°C. RNA quality was verified by agarose gel electrophoresis. First-strand cDNA was synthesized by oligo dT priming of 1 μg total RNA, using a superscript II RT kit (Invitrogen) according to the manufacturer's protocol. PCR amplification of cDNAs was performed in a total reaction volume of 50 μl that contained 1 μl of the RT reaction product, 0.25 μl of 5 units/μl Hot Gold Star DNA polymerase (Invitrogen), 5 μl of 10× PCR buffer, 1 μl of 10 mmol/l dNTPs, 4 μl of 25 mmol/l $MgCl_2$, and 1 μmol of each sense and antisense primer of the target gene. The following oligonucleotide primer sets were used: β1 integrin forward: GAGAGAGATTACTTCA GAC, reverse: AGCAGTCGTGTTACATTC; β4 integrin forward: GGGTCTTAT ACTGGGTGTAGG, reverse: CAGCTGACCCCTGTGGACT; laminin-5–γ2 chain forward: TGTACCCAGTGTTTCTGCTAT, reverse: CTCCTCTGTCCACGCGG TAG; and fibronectin forward: CCGGGTTCTGAGTACACAGTC, reverse: AGG GACCACTTCTCTGGGAGG. PCR amplification was initiated by one cycle of 95°C for 5 min followed by 35 sequential cycles of denaturation at 95°C for 45 s, annealing at melting temperature for 1 min, extension at 72°C for 2 min, and a final extension cycle at 72°C for 10 min in a thermocycler. As a negative control, the RT negative reaction product was added in the reaction. The presence of a single band amplified with specific primers for glyceraldehyde-3-phosphate dehydrogenase (forward: AATGCATCCTGCACCACC; reverse: GTAGCCATATTCATTGTCATA) or actin (forward: CGTGGGCCGCCCTAG-GCACCAG; reverse: TTGGCCTTAGGGTTCAGGGGGG) with the same cDNA was used as an internal control under identical conditions. Each experiment was repeated at least three times. PCR products were separated on Tris-acetate-EDTA 2% agarose gels containing ethidium bromide and photographed under UV illumination.

Immunohistochemistry. Pancreas sections (4-μm-thick) were cut from whole-tissue blocks and mounted on silane-coated glass slides. Paraffin sections were dewaxed, rehydrated with ethanol, and reacted with 5% hydrogen peroxide in methanol for 10 min to quench endogenous peroxidase activity. The sections were then treated with 0.1% trypsin for 30 min and incubated in 5% BSA for 15 min to block nonspecific binding of the antibody. After incubation for 1 h at room temperature with goat anti–laminin-5–γ2 diluted 1:100 in 5% BSA, biotinylated anti-goat IgG was applied for 30 min. Then, the slides were incubated with the peroxidase avidin-biotin complex for 30 min. The peroxidase reaction was developed in a diaminobenzidine tetrahydrochloride solution containing 0.01% hydrogen peroxidase. As a control, anti–laminin-5–γ2 antibody preadsorbed with laminin-5–γ2 peptide (Santa Cruz) or goat IgG were used instead of anti–laminin-5–γ2 antibody.

Data and statistical analysis. Data are the means ± SE for n independent experiments. Levels of significance for differences between groups were assessed by least significant difference ANOVA or by Student's t test for unpaired groups, using SPSS software.

RESULTS

Preferential attachment and spreading of β-cells on 804G matrix compared with vitronectin, fibronectin, or EHS laminin. As reported previously (22), β-cells adhere and spread on 804G matrix (Fig. 1*A*). To assess their attachment and spreading on other matrices, β-cells were seeded into Petri dishes coated with vitronectin, fibronectin, or laminin from basement membrane of EHS sarcoma cells. After 24 h, few cells were attached onto dishes coated with EHS matrix and vitronectin (Fig. 1*B–C*), whereas the majority of cells attached as clusters to dishes coated with fibronectin (Fig. 1*D*). However, cells were only found attached and well spread on dishes coated with 804G matrix.

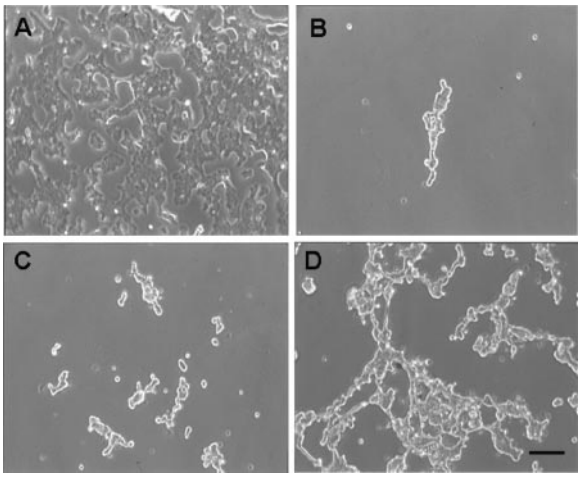

FIG. 1. β-Cells spread on 804G matrix only. Phase-contrast micrographs of β-cells cultured for 24 h on 804G matrix (*A*), EHS laminin (*B*), vitronectin (*C*), and fibronectin (*D*). Representative of three independent experiments. Bar = 100 μm.

Inhibitory effect of anti–laminin-5 antibody on β-cell spreading and insulin secretion. The expression of laminin-5 in 804G cells was confirmed by immunoblot analysis of 804G matrix. The CM6 antibody specifically detected a 160-kDa band corresponding to the α3 chain of laminin-5, and the 150-kDa band was stained with the anti–γ2 chain antibody. A 220-kDa band was also detectable by immunoblotting with anti-fibronectin antibody (Fig. 2). Then, to determine whether both laminin-5 and fibronectin are implicated in spreading of β-cells on 804G matrix, we used anti–laminin-5 and anti-fibronectin as blocking antibodies. β-Cells cultured on 804G matrix rapidly flattened and spread out, losing refringence compared with cells cultured on poly-L-lysine (Fig. 3*A*). Mouse IgG, used as control, did not affect the morphology of cells plated on either poly-L-lysine or 804G matrix. Anti–laminin-5 antibody did not interfere with attachment of cells on poly-L-lysine. However, spreading of β-cells on 804G matrix was significantly inhibited by anti–laminin-5 antibody compared with cells incubated without antibody. In the presence of anti–laminin-5 antibody, cell morphology was similar on poly-L-lysine and on 804G matrix. By contrast, anti-fibronectin antibody did not affect β-cell spreading on 804G matrix (Fig. 3*A*), whereas it completely inhibited attachment of cells on fibronectin-coated dishes, demonstrating its efficiency as a blocking antibody (data not shown). These results suggest that laminin-5, and not fibronectin, is an essential component of 804G in inducing β-cell spreading.

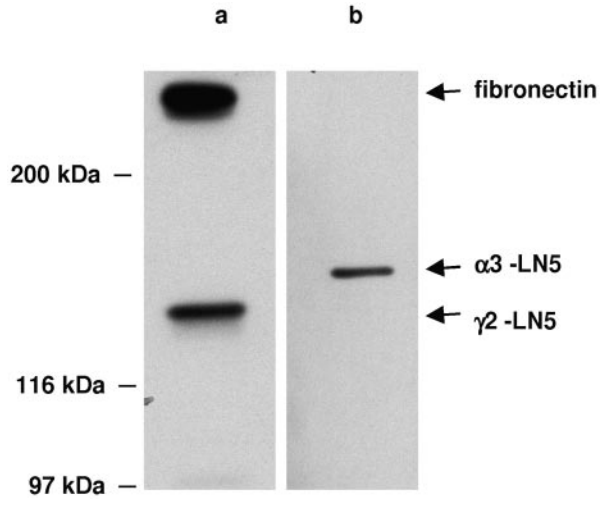

FIG. 2. Adhesion molecules in 804G matrix. 804G matrix was analyzed by Western blotting under reducing conditions with anti-fibronectin and anti–laminin-γ2 antibodies (*lane a*) or CM6 antibody (*lane b*). Arrows indicate the expected migration of proteins of interest. LN5, laminin-5.

To assess whether laminin-5 is essential for increased glucose-induced insulin secretion, β-cells were plated on 804G matrix previously treated with anti–laminin-5 antibody (CM6 antibody, 100 μg/ml) and then submitted to a static secretion test. 804G matrix, as expected, increased insulin secreted at 16.7 mmol/l glucose by twofold (P = 0.003) compared with poly-L-lysine (Fig. 3*B*). When 804G matrix was treated with CM6 antibody, stimulated insulin secretion was significantly reduced (P = 0.01) and was similar to that of β-cells attached on poly-L-lysine. Basal secretion was not affected by CM6 antibody. Anti-fibronectin antibody and control IgG did not affect either basal or stimulated insulin secretion (Fig. 3*B*). This result suggests that laminin-5 is involved in increased insulin secretion induced by 804G matrix.

β4 integrin is not expressed in islets. α3β1 and α6β1 integrins are receptors for laminin-5 and are known to be expressed by islet β-cells (22). α6β4 integrin is also a ligand for laminin-5, but its expression has not been studied so far in β-cells. 804G cells were used as a positive control. RT-PCR showed expression of β4 subunit in 804G cells but not in β-cells, whereas expression of β1 subunit was observed in both 804G cells and β-cells (Fig. 4*A*). By immunoblotting we confirmed that β-cells express β1 but not β4 integrin subunit and that both integrin subunits were expressed in 804G cells (Fig. 4*B*). These results suggest that only integrins of the β1 family are involved in β-cell binding to 804G matrix.

Inhibitory effect of anti–β1 integrin antibody on β-cell spreading and insulin secretion. To confirm that β1 integrins mediate adhesion of β-cells to 804G matrix, a monoclonal antibody against the β1 integrin subunit (Ha2/5) was tested as an inhibitor. By immunofluorescence, we confirmed the binding of this antibody on the surface of living β-cells (Fig. 4*C*). Then we analyzed the effect of Ha2/5 antibody on spreading of β-cells cultured for 5 h on poly-L-lysine or 804G matrix. β-Cell spreading on 804G matrix was significantly decreased when cells were incubated with Ha2/5 antibody (P = 0.035), whereas cells treated with hamster IgM were not affected (Fig. 5).

To test whether β1 integrins are involved in the effect of 804G matrix on insulin secretion, β-cells were attached for 2 h on Petri dishes coated or not coated with 804G matrix in the presence or absence of Ha2/5 antibody (1 or 2 μg/ml) and submitted to a static secretion test. As expected, 804G matrix induced a significant increase of glucose-stimulated insulin secretion compared with cells plated on poly-L-lysine (P = 0.02) (Fig. 6*A*). Ha2/5 antibody decreased stimulated insulin secretion of β-cells attached to 804G matrix (P = 0.016), so that no more difference was observed between poly-L-lysine and 804G (Fig. 6*A*). This antibody did not affect basal or stimulated insulin secretion of cells plated on poly-L-lysine (Fig. 6*A*). Similar results were obtained when insulin secretion from single cells was studied by reverse hemolytic plaque assay (Fig. 6*B*). These results suggest that β1 integrins are involved in the stimulatory effect of 804G matrix on insulin secretion

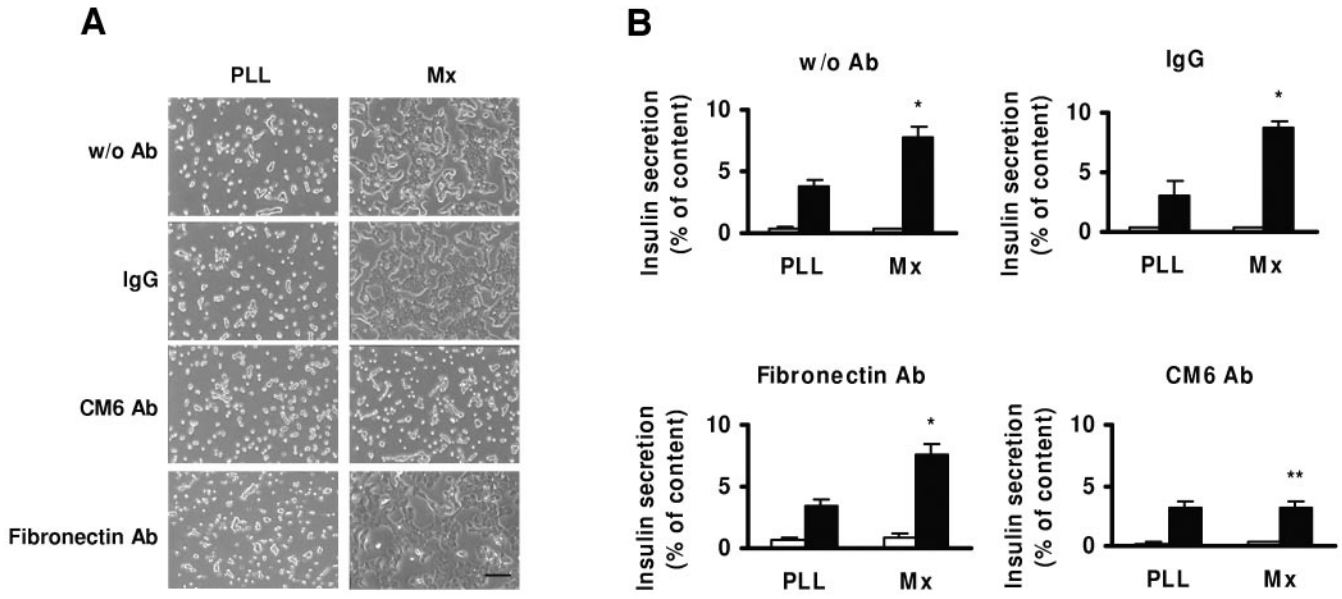

FIG. 3. Inhibitory effect of anti–laminin-5 antibody on β-cell spreading and insulin secretion. Petri dishes coated with poly-L-lysine or 804G matrix (Mx) were treated with anti–laminin-5 (CM6 antibody), anti-fibronectin (fibronectin antibody), mouse IgG, or PBS-BSA (without antibody). β-Cells were incubated on these dishes for 24 h. *A*: Phase-contrast microscopic views. Bar = 100 μm. *B*: Insulin secretion in response to 2.8 and 16.7 mmol/l glucose (n = 3–5). *P < 0.05 vs. poly-L-lysine (PLL) in respective condition; **P < 0.001 vs. 804G matrix control. Ab, antibody; w/o, without.

and that this effect persists even when secretion from single cells, deprived of intercellular contacts, are studied.

Blocking anti–β1 integrin antibody inhibits FAK phosphorylation. In a previous study, we showed that 804G matrix induces phosphorylation of FAK, a signaling protein that is activated on integrin ligand binding (37). Here, we studied the phosphorylation status of FAK in the presence of Ha2/5 antibody (Fig. 7). Adhesion of β-cells to 804G matrix resulted in a significant increase in FAK Tyr397 phosphorylation compared with adhesion to the control substrate poly-L-lysine (2.5-fold increase, P < 0.05). Blocking anti–β1 integrin antibody significantly decreased Tyr397 phosphorylation of FAK after adhesion to 804G matrix (86% inhibition compared with control cells adherent to 804G matrix, P = 0.03) but not to poly-L-lysine. β1 integrins are thus involved in 804G matrix–induced FAK phosphorylation of β-cells.

Assessment of extracellular matrix molecule expression in pancreatic islet. Having demonstrated that laminin-5 is implicated in 804G matrix–induced insulin secretion, we verified its expression in pancreatic islets. Expression of the characteristic γ2 chain of laminin-5 was studied by semiquantitative RT-PCR, Western blotting, and immunohistochemistry in rat and human islets. Results indicate that laminin-5, as well as fibronectin, are expressed in rat and human islets (Fig. 8*A*). Furthermore, when rat β-cells and non–β-cells were sorted, laminin-5–γ2 transcript was mainly observed in non–β-cells. However, fibroblastoid cells obtained by culturing non–β-cell fraction during several weeks did not express laminin-5 but, as expected, they expressed fibronectin (Fig. 8*A*). These results were confirmed at the protein level by Western blot (Fig. 8*C*). By immunohistochemistry, labeling of laminin-5–γ2 chain was observed in the endocrine cells at the periphery of the β-cell core in rat and human islets (Fig. 8*B*). These positive cells were shown to express glucagon, as determined by double immunostaining (data not shown). Furthermore, when the anti–laminin-5–γ2 antibody was preadsorbed with laminin-5–γ2 peptide or when nonimmune goat IgG was used instead of the

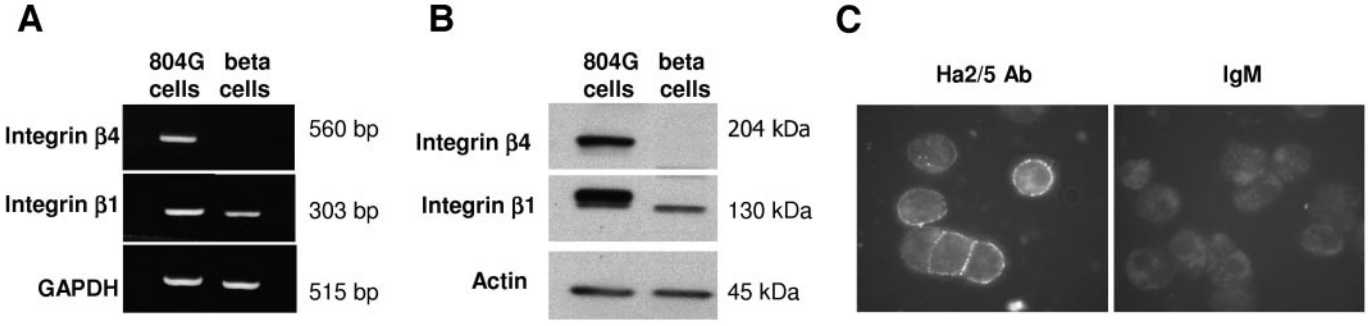

FIG. 4. β1 but not β4 integrin is expressed by β-cells. *A*: β4 and β1 integrin mRNA from β- or 804G cells was analyzed by RT-PCR with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as control. *B*: Proteins were analyzed by Western blotting using anti–β4 and –β1 integrin antibodies. Actin was used to confirm equal loading. *C*: Cells were incubated for 1 h in suspension with 2 μg/ml Ha2/5 antibody (Ab) or hamster IgM and attached into Cunningham chambers. Cells were then labeled with fluorescein isothiocyanate–conjugated anti-hamster antibody. Bar = 10 μm.

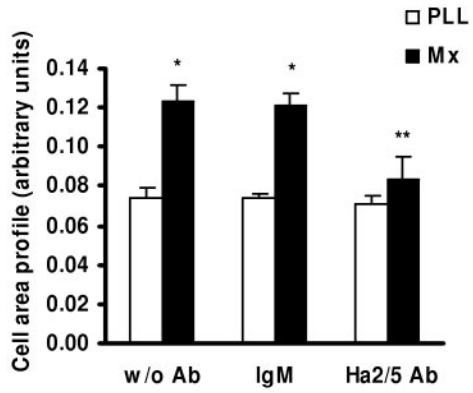

FIG. 5. Inhibitory effect of β1 integrin antibody on β-cell spreading. β-Cells in suspension were pretreated with 2 μg/ml Ha2/5 antibody (Ab), 2 μg/ml hamster IgM, or BSA (without antibody) for 1 h and then plated on poly-L-lysine (PLL)- or 804G matrix (Mx)-coated dishes in continuous presence of the antibody. Cells were fixed 5 h later. Cell area profile was measured, using ScionImage software. $n = 3–4$ with >100 cells analyzed per experiment. *$P < 0.02$ vs. poly-L-lysine control; **$P < 0.04$ vs. 804G matrix control. Ab, antibody; w/o, without.

anti–laminin-5–γ2 antibody, no laminin-5–γ2 labeling was observed.

DISCUSSION

We show here that anti–β1 integrin and anti–laminin-5 blocking antibodies interfere with spreading of β-cells on 804G matrix and with insulin secretion in response to glucose. These observations suggest that both β1 integrins and their cognate substrate, laminin-5, are important for the beneficial effect of 804G matrix on β-cell function.

A role of laminin-5 in β-cell function had been suggested by our previous work showing that 804G matrix–induced spreading of rat islet β-cells was prevented by anti–laminin-5 antibody (22). Furthermore, experiments performed with laminin-5 purified from 804G matrix suggest that this component is responsible for a pro-survival role on β-cells (37). Although these earlier studies suggested that laminin-5 could be implicated in spreading and increased survival of β-cells on 804G matrix, it remained unclear whether this also holds true for increased insulin secretion on this matrix. We now not only confirm that laminin-5 is implicated in β-cell spreading, but we also show that this protein contributes to increased glucose-stimulated insulin secretion on 804G matrix. It remains to be established whether this is caused by direct signaling from the matrix to the exocytotic machinery and/or pathways that regulate exocytosis or by indirect effects perhaps secondary to increased cell spreading or decreased apoptosis.

A continuous periinsular extracellular matrix that contains laminin, fibronectin, and collagen types IV and V has been described in human pancreas (21,38). Other studies have shown that basement membrane structure and major extracellular matrix proteins are not found between islet cells (11,38,39). Within the islet, basement membrane was observed only around capillaries (21). In humans, collagen IV was detected in association with the islet microvasculature (40). Intimate relationships between individual β-cells and islet capillaries, and direct contact between β-cells and subendothelial basement membrane, have been confirmed by electron microscopy studies (41,42). In human pancreas, basement membrane of duct cells showed immunoreactivity for ladsin, a homolog of laminin-5, but in this study, islets were not analyzed (43). The expression and localization of laminins are poorly documented, and the presence of laminin-5 in islets has not been reported previously. In this study we demonstrated that laminin-5 is expressed in rat and human islets, mainly

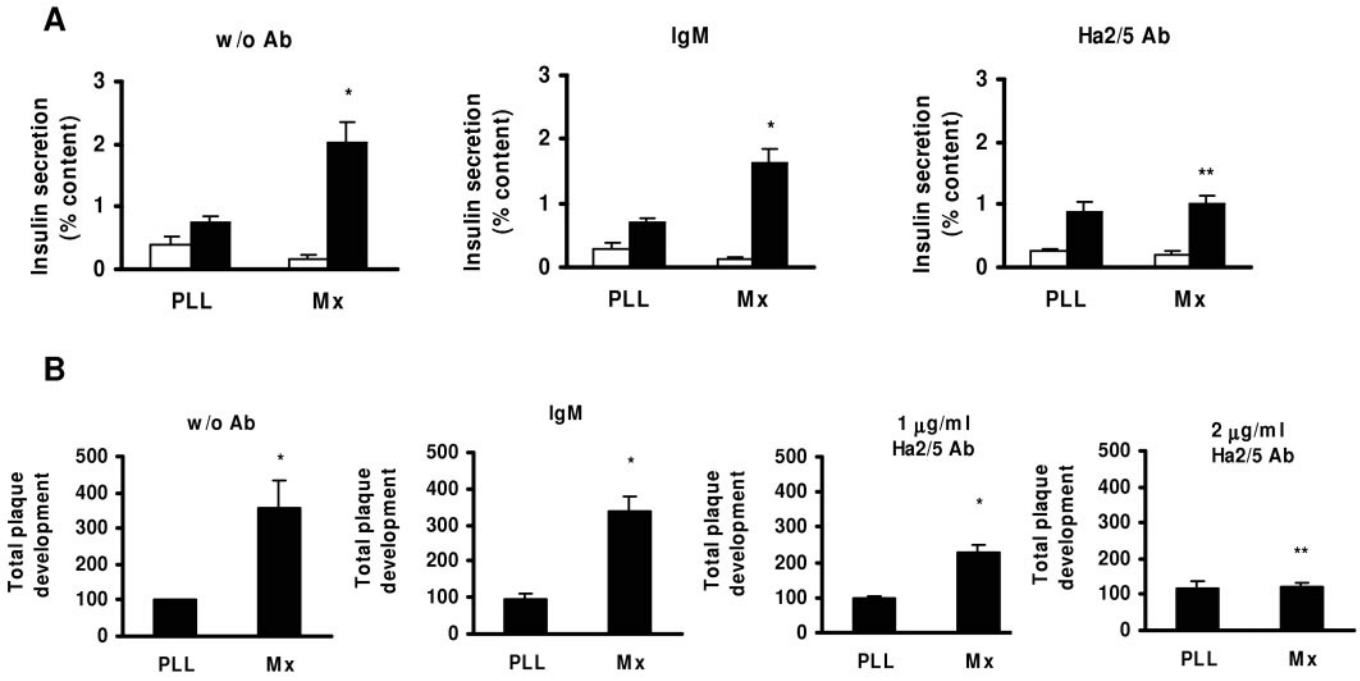

FIG. 6. Inhibitory effect of β1 integrin antibody on insulin secretion in response to glucose. β-Cells were treated as described in Fig. 5. *A*: After 2 h of culture, insulin secretion was measured during successive 1-h incubations at 2.8 and 16.7 mmol/l glucose. *$P < 0.02$ vs. poly-L-lysine (PLL) control; **$P < 0.001$ vs. 804G matrix (Mx) control. *B*: Single-cell insulin secretion measured by reverse hemolytic plaque assay after 1 h at 16.7 mmol/l glucose without Ha2/5 antibody (Ab; 1 or 2 μg/ml). Secretion is expressed as total plaque development (average plaque area × proportion of plaque-forming cells), $n = 3$. *$P < 0.02$ vs. poly-L-lysine control; ** $P < 0.001$ vs. 804G matrix control. w/o, without.

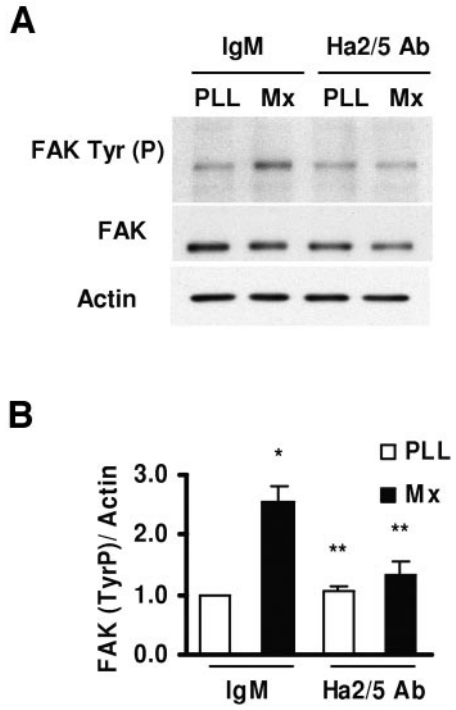

FIG. 7. Inhibitory effect of β1 integrin antibody on 804G matrix (Mx)-induced FAK phosphorylation. *A*: Lysates of β-cells attached on poly-L-lysine (PLL) or 804G matrix for 4 h in the presence or absence of Ha2/5 blocking antibody (Ab) were immunoblotted with anti–phospho-FAK (Tyr-397) and anti-actin antibodies. Then, the immunoblot was stripped and reprobed for total FAK protein. Representative results are shown from one experiment *B*: Band intensities (phospho-FAK [Tyr-397]/actin), $n = 3$. *$P < 0.01$ vs. poly-L-lysine IgM; **$P < 0.03$ vs. 804G matrix IgM. FAK Tyr(P), phospho-FAK (Tyr-397).

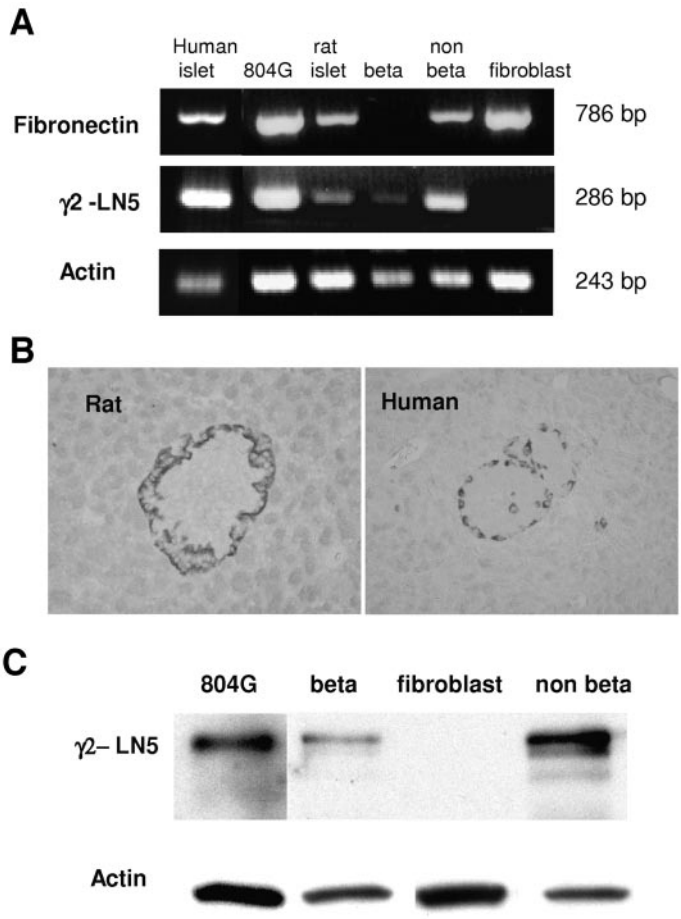

FIG. 8. Laminin-5 is mainly expressed by non–β-cells in pancreatic islets *A*: Laminin-5 and fibronectin mRNA were analyzed in human and rat islets, β-cells and non–β-cells and in 804G cells by RT-PCR. Actin was used as control. *B*: Immunohistochemical staining of laminin-5–γ2 chain (γ2-LN5) in rat and human pancreas. *C*: Laminin-5 expression was analyzed in rat islets, β-cells, and non–β-cells and in 804G cells by Western blot. Actin was used as control.

in α-cells. This is in agreement with data showing that in some circumstances, α-cells may improve the function of β-cells (44), and this could be attributable to provision of a more appropriate extracellular matrix as well as possible paracrine effects of glucagon. Moreover, laminin-5 was also detected in islet culture media (data not shown), suggesting this molecule could be secreted by insular cells. Whether laminin-5 is part of the basal lamina depositing under islet endocrine cells remains to be demonstrated. Whatever laminin isoform is expressed in islets in vivo, it is reasonable to expect that this isoform could play a similar role in activating β1 integrin receptors on β-cells as laminin-5 does in vitro.

Integrins are a large family of adhesion receptors that mediate cell-matrix and cell-cell adhesion (45). Our group has shown that rat pancreatic β-cells express α6β1 and α3β1 integrins (22,29), which are receptors for laminin-5. α6β4 integrin is another well-known receptor of laminin-5. Here, we show by RT-PCR and Western blot that β4 subunit integrin is not expressed in rat β-cells. This is in agreement with previous work showing that human β-cells express mRNA for the integrin subunits α3, α5, α6, and β1 but not β4 (46). It was shown previously that function-blocking antibodies directed against the α6 integrin subunit blocked cell spreading induced by 804G matrix (22). The expression of this α6 integrin subunit is regulated by glucose and correlates with insulin expression (22). However, the anti–α6 integrin blocking antibody does not affect insulin release (D.B., unpublished observations). This is expected because other integrin subunits known to partner β1, such as α3, α5, and αv, have been shown to be expressed on β-cells and may play a role in insulin secretion (47). Here, we used a blocking β1 integrin subunit antibody that interferes with all β1 integrin heterodimers, and we showed that this prevented both the spreading and the improved insulin secretion induced by 804G matrix. These results suggest that engagement of β1 integrins by laminin-5 plays an important role in the maintenance of optimally regulated β-cell function. This subunit associates with different α-subunits, and thus more than one β1 integrin heterodimer might participate in this phenomenon. These data are the first to implicate laminin-integrin binding in the regulation of insulin secretion and spreading of purified and isolated adult β-cells. It has been previously reported that α1β1 and collagen IV contribute to β-cell functions known to be important for islet morphogenesis (40). Furthermore, the β1 family of integrin has been shown to be involved in early pancreatic developmental events (47,48), including differentiation and maturation of islets (6).

There is evidence that on integrin activation, a cascade of protein phosphorylations on tyrosine residues activates intracellular signaling pathways (49). FAK was identified as a tyrosine-phosphorylated protein that localized at focal contacts and acts downstream of growth factor and inte-

grin receptors. We showed previously that 804G matrix induces phosphorylation of FAK (37). Here, we demonstrated that the function-blocking antibody directed against β1 integrin subunit inhibited phosphorylation of FAK induced by 804G matrix. The treatment of cells with an equal amount of IgM antibody did not compromise β-cell spreading, insulin secretion, and FAK phosphorylation, indicating that the interference with β1 function by blocking antibody is not the result of nonspecific interactions.

In summary, our study shows that the function of dispersed primary pancreatic β-cells can be maintained by attachment to 804G matrix in vitro. This maintenance of an optimally regulated β-cell function is mediated by β1 integrin–laminin-5 interaction. It is concluded that outside-in signaling via engagement of β1 integrins by laminin-5 is an important component of normal β-cell function.

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