Islet Endothelial Cells Induce Glycosylation and Increase Cell-surface Expression of Integrin β1 in β Cells*

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Background: Islet endothelial cells induce pseudoislet formation in β cells and ECM deposition.

Results: Pseudoislet formation results in glycosylation and increased cell surface expression of integrin β1.

Conclusion: Posttranslational modifications of integrins in β cells are a novel mechanism for controlling β-cell function.

Significance: Alternative integrin β1 glycosylation represents a novel mechanism that may control β cells in health and in disease.

The co-culturing of insulinoma and islet-derived endothelial cell (iEC) lines results in the spontaneous formation of free-floating pseudoislets (PIs). We previously showed that iEC-induced PIs display improved insulin expression and secretion in response to glucose stimulation. This improvement was associated with a de novo deposition of extracellular matrix (ECM) proteins by iECs in and around the PIs. Here, iEC-induced PIs were used to study the expression and posttranslational modification of the ECM receptor integrin β1. A wide array of integrin β subunits was detected in BTC3 and NIT-1 insulinomas as well as in primary islets, with integrin β1 mRNA and protein detected in all three cell types. Interestingly, the formation of iEC-induced PIs altered the glycosylation patterns of integrin β1, resulting in a higher molecular weight form of the receptor. This form was found in native pancreas but was completely absent in monolayer β-cells. Fluorescence-activated cell sorting analysis of monolayers and PIs revealed a higher expression of integrin β1 in PIs. Antibody-mediated blocking of integrin β1 led to alterations in β-cell morphology, reduced insulin gene expression, and enhanced glucose secretion under baseline conditions. These results suggest that iEC-induced PI formation may alter integrin β1 expression and posttranslational modification by enhancing glycosylation, thereby providing a more physiological culture system for studying integrin-ECM interactions in β cells.

The islets of Langerhans are three-dimensional structures containing insulin-producing β cells (1). Disruption of the islet structure alters β-cell function by inducing dedifferentiation and impairing cell survival (2–4). Three-dimensional β-cell aggregates, or pseudoislets (PIs),2 are useful for the study of β-cell biology (5–7). We previously developed a novel method for the spontaneous induction of free-floating PIs using co-cultures of insulinoma cells and islet-derived endothelial cells (iECs) (8). The formation of iEC-induced PIs improved β-cell function and was associated with a de novo deposition of key extracellular matrix (ECM) proteins.

ECM proteins are produced by iECs and play a critical role in β-cell function and mass (9). This effect is partially mediated by the ability of iECs to produce pro-β-cell factors (10) and support islet structure (11, 12). In isolated human islets, ECM proteins delay β-cell dedifferentiation and maintain insulin expression over time (13). ECM proteins are recognized by integrins, proteins consisting of α and β subunits, with integrin β1 playing an important role in β-cell function. Integrin β1-mediated signaling can enhance the survival of adult rat β-cells as well as the survival and differentiation of human fetal islet cells ex vivo (14, 15). Inversely, functional blockage of integrin β1 results in decreased binding to ECM ligands and increased islet cell apoptosis (16–19). The role of integrins in cell survival, function, and death can be mediated by their ability to bind non-ECM components such as soluble growth factors and matrix metalloproteases (20).

Here we examined the expression of eight integrin β subunits in murine insulinoma cell lines and primary islets of human origin, demonstrating the expression of integrin β1 in all cell types. We show that PI formation induces the higher molecular weight native form of integrin β1 by protein glycosylation. This modification of integrin β1 glycosylation is dependent on the presence of iECs and results in an increase in cell surface integrin β1 expression. Blockage of integrin β1 decreases insulin gene expression and disrupts insulin release in response to increased glucose concentrations.

Experimental Procedures

Reagents—Dulbecco’s modified Eagle’s medium, penicillin/streptomycin/neomycin mixture, and amphotericin B were from Life Technologies. Fetal bovine serum (FBS) was from Atlanta Biologicals (Lawrenceville, GA). Radioimmune precipitation assay buffer, protease inhibitor mixture, and ECL Western blotting chemiluminescent substrate for detecting horseradish peroxidase (HRP) were from Thermo Scientific (Rockford, IL). PNGase F was from New England BioLabs (Ipswich, MA). PVDF membrane (0.2 μm pore size) and blot-
The integrin β1 maturation of the islet

**List of primers used for quantitative RT-PCR analysis**

| Gene     | Primer sequences |
|----------|------------------|
| Iggb1    | F-ACCACCGCCCGCCAGACCTT | R-CAGGAAACCGTGGTCACAAPTC |
| Iggb2    | F-AGCTGGCCACAACACTTTCC | R-TGCTCGAATGCGGATGATCT |
| Iggb3    | F-AGTTGGCGCGAACAACTCTT | R-CCTCTTGGTTGAGTACAGAP |
| Iggb4    | F-ATTCTGGACAGATACTGCCAATGCC | R-AGAAGACTGACCTCCCTCC |
| Iggb5    | F-TGAGTCGAGAGATCTCAGG | R-AGATGCACTCCTCAAGGCTC |
| Iggb6    | F-TGCTGCGCCACACAGACAG | R-AGCTGAGAAAGATGTTTCCCA |
| Iggb7    | F-GAGGCGCTCTCCCTTCC | R-AACCTGCTCTCCAGTCC |
| Iggb8    | F-AGGCTCTCCCTTCC | R-TCCCTGTACCAATGAAGTCT |
| Actb     | F-GAAAAGGCTGTTAAGCAGAACG | R-AAGAAAGGCTGTTAAGCAGAACG |
| Ins1     | F-CCCTGGAGCTGTTAAGCAGAACG | R-AGAAGAAGGCTGTTAAGCAGAACG |
| Pdx1     | F-CCCTGGAGCTGTTAAGCAGAACG | R-AGAAGAAGGCTGTTAAGCAGAACG |
| MafA     | F-GGAAGGCTGTTAAGCAGAACG | R-AGAAGAAGGCTGTTAAGCAGAACG |

*E, forward; R, reverse.*

**Analysis**

The non-tissue culture Petri dishes were used to minimize islet adhesion to the dish surface.

**Reverse Transcription and Real-time PCR**

Total RNA was purified from cell lysates with an RNaseasy Mini Kit (Qiagen, Valencia, CA) and reverse-transcribed with a Transcriptor High Fidelity cDNA Synthesis kit (Roche Applied Science) and a MyCycler thermal cycler (Bio-Rad) under the following conditions: RNA denaturation at 65 °C for 10 min, cooling at 4 °C for 5 min with the addition of the reverse transcriptase mix, cDNA synthesis at 45 °C for 30 min, and reverse transcriptase inactivation at 85 °C for 5 min. Target cDNA was amplified using a CFX96 real-time system (Bio-Rad) and a SYBR Green I Master reaction mix (Roche Applied Science). Real-time PCR conditions were as follows: preincubation at 95 °C for 5 min for TaqDNA polymerase activation and DNA denaturation, 45 cycles of DNA denaturation at 95 °C for 1 min, primer annealing at 67 °C (for insulin) or 60 °C (all other genes) for 30 s, and target elongation at 72 °C for 45 s. β-Actin was used as an internal control. See Table 1 for a complete list primers used for amplification.

**Immunofluorescence Staining and Confocal Imaging**

Insulinoma monolayers were grown on glass bottom culture dishes (MatTek, Ashland, MA) coated with poly D-lysine. Monolayers were washed with PBS, fixed with paraformaldehyde (2% in PBS) for 10 min and permeabilized with Triton X-100 (0.25% in PBS) for an additional 10 min. Free-floating PIs were washed, fixed, and permeabilized in suspension. Donkey serum (5% in PBS) was used as a blocking agent (1 h at room temperature) and antibody diluent. Cells were incubated with primary antibodies for 1 h at 37 °C and then washed twice with PBS. After incubation with fluorochrome-labeled secondary antibodies for 30 min at room temperature, cells were washed two more times and stained with DAPI. Samples processed without primary antibodies served as nonspecific staining controls. Stained cells were analyzed using a Nikon Eclipse Ti confocal microscope (Nikon, Melville, NY).
Western Blotting—Cells were lysed in radioimmunoprecipitation assay buffer (Pierce) supplemented with protease inhibitors. Lysates were subjected to reducing and denaturing SDS-PAGE. Samples treated with PNGase F to cleave N-linked glycans received enzyme treatment for 1 h at 37°C before gel electrophoresis. Separated proteins were electrophoretically blotted onto a PVDF membrane under wet transfer conditions for 1 h at 100 V. Nonspecific membrane binding sites were blocked for 1 h at room temperature with 5% nonfat dry milk in PBST followed by overnight incubation at 4°C with primary antibody diluted in the same blocking solution. Primary antibody was washed away with PBST, and the membrane was incubated for 1 h at room temperature with HRP-conjugated secondary antibody (1% nonfat dry milk in PBST). The membrane was thoroughly washed again, soaked in HRP substrate solution for 1 min, and exposed to autoradiography film for visualization of target protein bands.

FACS Staining—Evaluation of integrin β1 positivity in insulinoma monolayers and PIs was done by fluorescence-activated cell sorting (FACS) analysis using a C6 flow cytometer (BD Biosciences). Suspensions of dispersed monolayer and PI cells were washed with PBS and fixed with 2% paraformaldehyde for 10 min to avoid integrin β1 internalization. Fixed cells were washed twice more and suspended in FACS buffer containing either anti-integrin β1 antibody or isotype control (Abcam). Cells were stained with FITC-conjugated secondary antibody (Jackson ImmunoResearch) and analyzed using an Accuri FACS analyzer (BD Biosciences).

Glucose-stimulated Insulin Secretion—After reaching ~80% confluency in a 96-well tissue culture plate (BD Biosciences), NIT-1 monolayers were starved overnight in 5.5 mM glucose medium. PIs were starved in a Petri dish and dispensed in a non-tissue culture plate before GSIS. For some experiments, starvation was carried out in the presence of either anti-integrin β1 antibody or isotype control for 24 h. Monolayers and PIs were washed twice with 2 mM glucose Krebs-Ringer bicarbonate HEPES buffer and then incubated for 1 h with antibodies diluted in the same Krebs-Ringer bicarbonate HEPES buffer. Antibodies were subsequently removed, and GSIS was carried out for 1 h with either 0 or 16 mM glucose Krebs-Ringer bicarbonate HEPES buffer. Secreted insulin was quantified from the cell supernatants using an immunoassay kit (Crystal Chem Inc., Downers Grove, IL) according to the manufacturer’s instructions.

Statistical Analysis—Data are expressed as the means ± S.E. Differences between means were analyzed by unpaired t test using Prism 5 (GraphPad software) or analysis of variance and were considered statistically significant when p < 0.05.

Results

Integrin β Subunits Are Expressed at Variable Levels in Insulinoma Cell Lines—Integrin β subunits are critical components of integrin receptor complexes. Therefore, we examined the mRNA levels of all eight mammalian integrin β subunits in two distinct murine insulinoma lines, NIT-1 (Fig. 1A) and βTC3 (Fig. 1B), and in primary C57BL/6 mouse islets (Fig. 1C). Integrin β8 was undetected in all samples (Fig. 1, A–C), whereas integrin β5 mRNA expression was the highest of all integrin β chains. (Fig. 1, A–C, insets). Integrin β1 mRNA was readily detected in both βTC3 and NIT-1 cells as were integrins β2, -3, -4, -6, and -7.
Previous reports suggest a critical role for integrin β1 in mediating normal β-cell function (14, 16, 17, 23–28). The cellular distribution of integrin β1 was examined in βTC3 and NIT-1 monolayers grown on glass-bottom dishes, fixed, and stained using anti-integrin β1 antibody. Both monolayer cell lines showed a strong staining for integrin β1. Z-stack confocal imaging of top and bottom view plains revealed an accumulation of integrin β1 on the bottom surface of the cells in contact with the glass surface (βTC3 (Fig. 1D) and NIT-1 cells (data not shown)). Integrin β1 displayed a punctuated distribution in each insulinoma cell type. These data demonstrate a wide expression of various integrin β chain members in insulinomas and primary islets, with integrin β1 accumulating at the point of adhesion in insulinoma cells.

Integrin β1 Is Evenly Distributed in NIT-1 PIs—We have previously reported a rapid method for the formation of PIs using a co-culture system of iECs and βTC3 insulinoma cells (8). Here, we examined the ability of a second insulinoma line, NIT-1, to form PIs when cocultured with MS1 cells. Although NIT-1 cultured alone grew as monolayers, NIT-1 cultured in the presence of MS1 cells formed cellular aggregates as early as 24 h post co-culture (Fig. 2A). By 48 h the PI aggregates grew in size and assumed a spheroid appearance. Spontaneous detachment of free-floating PIs was observed as early as 3 days (Fig. 2A). This detachment was accompanied by an increase in PIs numbers and size after 1 week in co-culture (Fig. 2A).

A hallmark of PI formation using our co-culture system is the deposition of the ECM proteins col-IV and laminin (8). Z-stack confocal imaging of 7-day-old PIs showed intense insulin, col-IV, and laminin staining (Fig. 2B). Both col-IV and laminin were detected throughout the PI (1). Integrin β1 was readily detected in NIT-1 PIs (Fig. 2C). In contrast to the punctuated staining of integrin β1 in monolayer cells, PIs showed a unified distribution in a three-dimensional structure. FACS analysis of dispersed NIT-1 PI cells failed to detect MS1 cells in the PI structure (Fig. 2D), suggesting that NIT-1 PIs consisted of β-cells only. Overall, these data demonstrate the ability of iECs to induce PI formation, ECM deposition, and integrin β1 expression in insulinomas.

Integrin β1 Glycosylation and Protein Expression Levels Are Altered after PI Formation—Integrin receptors are key players in the recognition of ECM proteins by β-cells. Therefore, we examined whether PI formation can alter integrin β1 expres-
Integron and posttranslational modifications in NIT-1 and βTC3 insulinomas. Western blotting analysis showed a single integrin β1 band at 105 kDa in monolayer cells (Fig. 3A). In contrast, both NIT-1 and βTC3 PIs showed an additional band at 125 kDa, similar to the bands detected in C57BL/6 pancreas (Fig. 3A, black arrows). Integrin function and expression are controlled, in part, by posttranslational modifications (29). Thus, we assessed whether the difference in integrin β1 protein size between PIs and monolayer cells was due to posttranslational modifications. Cell lysates from monolayers, PIs, and pancreas were treated with the N-deglycosylating enzyme PNGase F. PNGase F treatment produced a single deglycosylated band at ~88 kDa for monolayers and PIs and native pancreatic tissue (Fig. 3A). Densitometry analysis of Western blot staining showed similar levels of integrin β1 expression between NIT and βTC3 monolayers and PIs (Fig. 3B). Glycosylation can alter integrin β1 cell surface expression (30, 31). Therefore, intact monolayers and dispersed PIs cells were stained for integrin β1 expression. FACS analysis revealed higher levels of integrin β1 on the surface of NIT-1 cells from PIs when compared with monolayers (Fig. 3C, mean fluorescence intensity: monolayer = 181 × 10^3 ± 4.3 × 10^2, PIs = 405 × 10^3 ± 4.8 × 10^2, p < 0.026) with the overall percentage of integrin β1-positive cells PIs increasing as well (Fig. 3D, monolayer = 18%, PIs = 37%, p < 0.05). Similar results were seen with βTC3 insulinomas (data not shown).

The altered glycosylation of integrin β1 in PIs led us to examine whether protein glycosylation of other members of the integrin family was also modified in PIs. Western blot analysis failed to detect the expression of αV in both insulinoma lines (Fig. 4). In contrast, integrins α1 and α3 were readily detected in monolayer and PIs. However, the formation of PIs failed to induce differential glycosylation in these integrin chains (Fig. 4). Western blot analysis of integrin α6 showed increased protein size in PIs compared with monolayers, suggesting that PI formation can increase integrin α6 glycosylation in β-cells (Fig. 4).

In summary, these data demonstrate for the first time the presence of an alternative posttranslational pathway of the integrin β1 receptor in insulin-producing cells after the formation of PIs. These changes result in increased expression of cell surface protein in β-cells. Integrin β1 expression is associated with the expression of some integrin α chains known to form heterodimers with integrin β1.

![Figure 3. PI formation is associated with increased integrin β1 expression and protein maturation by glycosylation.](image)
Anti-integrin β1 Antibody Alters Monolayer and PI Morphology, Insulin Gene Expression, and GSIS—To examine the effects of integrin β1 blockade on β-cell structure and function, NIT-1 and βTC3 insulinoma monolayers and PIs were incubated either with anti-integrin β1 antibody directed against the extracellular domain of the receptor or with an isotype control. Anti-integrin β1 antibody, but not isotype control, induced a marked change in β-cell morphology in both monolayers and PIs (Fig. 5A). This morphological change was previously reported during blocking of integrin β1 function (32, 33). Next we examined the effects of acute integrin β1 blockade on GSIS in monolayer NIT-1 cells. Interestingly, a 1 h incubation with anti-integrin β1 antibody resulted in a 2-fold increase in baseline insulin secretion when compared with isotype-treated cells, whereas the response to a 16 mM glucose stimulation remained unchanged (Fig. 5B, insulin levels; isotype-treated: 0 mM glucose = 166.3 ± 7.0 ng/mg, 16 mM glucose = 638.8 ± 70.3 ng/mg; anti-integrin β1: 0 mM glucose = 344.1 ± 30.9 ng/mg, 16 mM glucose = 597.1 ± 64.0 ng/mg; p < 0.005 isotype control versus anti-integrin β1 at 0 mM glucose). A 24 h exposure to anti-integrin β1 antibody resulted in a statistically significant decrease in insulin gene expression in both βTC3 and NIT-1 cells (Fig. 5C; NIT-1, ~61% decrease in expression by anti-integrin β1 antibody versus isotype control, p < 0.014; βTC3, ~72% decrease in expression by anti-integrin β1 antibody versus isotype control, p < 0.0026). GSIS of monolayer NIT-1 cells revealed a defect in their ability to respond to high glucose stimulation when incubated in the presence of anti-integrin β1 antibody (Fig. 5C; insulin levels; isotype-treated: 0 mM glucose = 22.7 ± 5.2 ng/mg, 16 mM glucose = 212.5 ± 13.7 ng/mg; anti-integrin β1 antibody: 0 mM glucose = 23.65 ± 2.5 ng/mg, 16 mM glucose = 112.4 ± 12.3 ng/mg; p < 0.0055 isotype control versus anti-integrin β1 antibody at 16 mM glucose). The reduction in insulin expression and secretion was associated with reduced expression of Pdx1 and MaFA (Fig. 5D; Pdx1, ~39% decrease in expression by anti-integrin β1 antibody versus isotype control, p < 0.017; MaFA, ~44% decrease in expression by anti-integrin β1 versus isotype control, p < 0.0448). Taken together, these data demonstrate a critical role for integrin β1 in maintaining normal β-cell morphology, insulin gene expression, and the regulation of insulin release under baseline conditions and after exposure to high glucose levels.

Disruption of Islet Structure and/or Removal of iECs from the Culture Can Reverse Integrin β1 Glycosylation—The stimulation of integrin β1 glycosylation in β-cells by iECs and the importance of integrin signaling in mediating normal β-cell function prompted us to examine whether the glycosylation can be reversed upon islet disruption or the removal of PIs from MS1 cells. To this end, NIT-1 and βTC3 insulinomas were divided into four groups. Group 1 was composed of PIs collected immediately after an 8-day co-culture with MS1. Group 2 served as a control for β-cells grown as monolayers. Group 3 consisted of 8-day-old PIs removed from co-cultures and grown in suspension for an additional 8 days. Group 4 also consisted of isolated PIs but was allowed to adhere and grow on a tissue culture-treated surface to induce the disruption of PI structure. Phase contrast imaging of reattached PIs (Group 4) showed a completed loss of PI structure and the reappearance of monolayer configuration (Fig. 6A). In contrast, PIs grown in suspension in the absence of iECs (Group 3) showed normal PI architecture with minimal to no attachment to the culture surface (Fig. 6A). Western blot analysis of integrin β1 revealed a near complete loss of the 125 kDa glycosylated form in both Group 3 and Group 4 PIs when compared with PIs from Group 1 (Fig. 6B), with reattached NIT-1 cells showing a more profound loss of glycosylation. Despite this change in glycosylation, the size of integrin β1 in Groups 3 and 4 remained at a slightly higher molecular weight than monolayer cells (Group 2). The plasticity of integrin β1 glycosylation raised the question of whether a similar phenomenon also occurs in primary human islets (hIslets) under the conditions of prolonged culture. To test this hypothesis, primary hIslets were divided into two groups. Group 1 of hIslets harvested on the day of delivery and used as a control for native integrin β1 glycosylation, whereas Group 2 of hIslets were cultured for 14 days in suspension. Phase contrast imaging showed minor differences in islet morphology between Group 1 and Group 2 even after 2 weeks in culture (Fig. 6C). Western blot analysis of both groups revealed the expression of a high molecular weight (~140 kDa) and low (~110 kDa) molecular weight isoforms of integrin β1, similar to those seen in murine PIs. However, prolonged culturing of hIslets resulted in a near complete loss of the 140 kDa band in all three replicates, with the lower 110 kDa isoform remaining intact (Fig. 6D). These changes in integrin β1 protein size suggested that alternative glycosylation of integrin β1 may exist in human islets. PNGase F treatment of protein lysates from hIslets verified a hyperglycosylated form of integrin β1 at the 140 kDa size, as seen in murine PIs and pancreas (Fig. 6E). Overall, the plasticity of the integrin β1 posttranslational modification, the correlation between glycosylation and islet structure, and the need for iEC to maintain integrin β1 protein glycosylation appear to exist in both murine and human cells.

Discussion

The co-culturing of β-cells together with iECs induces the formation of three-dimensional PIs with improved β-cell function and the de novo deposition of key ECM proteins (8). Integrin β1 Maturation in the Islet
Integrin β1 Maturation in the Islet

Integrins can directly bind to the ECM. In this report, we examined the expression of different integrin β subunits in insulinoma monolayers, PIs, and primary islets. We show that integrin β1 mRNA and protein are readily detected in all cell types. PI formation leads to the maturation of integrin β1 protein glycosylation similar to that seen in native pancreas and in mouse islets.

FIGURE 5. Blockage of integrin β1 on β-cells alters cell conformation, reduces insulin gene expression, and dysregulates insulin secretion. A, microscope images of PIs and monolayers treated for 24 h with anti-integrin β1 antibody or isotype control. B, quantitative real-time-PCR analysis of insulin gene expression in NIT-1 and βTC3 insulinoma cells incubated with anti-integrin β1 antibody or the respective isotype control for 24 h. Data are normalized to actin levels and represent an average of three independent repeats. C, insulin ELISA analysis of NIT-1 GSIS. Cells were stimulated with 16 mM glucose for 1 h in the presence or absence of anti-integrin β1 antibody. Amounts of secreted insulin were normalized to total cellular protein content. Data represent an average of three independent repeats. *, p < 0.05; **, p < 0.003. D, quantitative real-time-PCR analysis of Pdx1 and MafA gene expression after 24 h of incubation with anti-integrin β1 antibody or the respective isotype control. Data are normalized to actin levels and represent an average of three independent repeats.
This maturation of integrin $\beta 1$ can be reversed by PI disruption, whereas the blockage of integrin $\beta 1$ alters cell morphology reduces insulin gene expression and results in the dysregulation of insulin secretion under basal glucose levels and upon glucose stimulation. Similar glycosylation patterns are also observed in primary hIslets, with prolonged culturing leading to a loss of hyperglycosylated integrin $\beta 1$.

PIs generated from co-cultures of iECs and $\beta T C 3$ insulinoma cells are rich in ECM protein deposition (8). Here we showed that the critical ECM components col-IV and laminin are also identified in and around iEC-induced NIT-1 PIs, suggesting an innate ability of iECs to deposit ECM proteins onto various insulinoma cell lines. Our examination of the gene expression of all eight integrin $\beta$ family members revealed variable levels of integrin $\beta 1$–7 mRNA. Interestingly, although integrin $\beta 5$ and $\beta 7$ mRNA was expressed in the highest amount, protein expression of these $\beta$ chains was extremely low (data not shown). In contrast, integrin $\beta 1$ protein levels were high in insulinoma cell lines, mouse pancreas, and primary hIslets, supporting previous findings that identified integrin $\beta 1$ as a receptor in $\beta$ cells (16, 17, 23–28).

The integrin $\beta 1$ subunit associates with 12 different $\alpha$ subunits, forming a myriad of receptors capable of interacting with ECM proteins (34). For example, integrin $\alpha V \beta 1$ and $\alpha 1 \beta 1$ control human fetal $\beta$-cell attachment and motility on vitronectin and col-IV substrates, respectively, and promote insulin secretion (16, 17). Similar effects were shown via col-1-$\alpha 3 \beta 1$ and laminin-5-$\alpha 6 \beta 1$ interactions (23–25). Inversely, $\beta$-cell dedifferentiation can be mediated by integrin $\alpha 1 \beta 1$ as human fetal $\beta$-cells cultured on col-IV exhibited decreased insulin gene expression (27). Studies conducted with isolated human fetal/ adult islet cells and INS-1 cells cultured on col-I and -IV substrates showed that integrin $\alpha 3 \beta 1$ is an important regulator of cell proliferation and function (26, 28). Our examination of integrin $\alpha$ subunits in monolayers and PIs showed variable differences in protein glycosylation and expression levels between the two cell culture systems. For example, both NIT-1 and $\beta T C 3$ showed active expression of $\alpha 1$, $\alpha 3$, and $\alpha 6$, demonstrating the potential for several integrin $\beta 1/\alpha$ combinations. Integrin $\alpha 3$ has been long recognized as a key integrin chain in the control of $\beta$-cell function, the activation of Pdx1, and the maintenance of $\beta$-cell differentiation. Our Western blot analysis supports these previous findings, as integrin $\alpha 3$ was expressed at the highest levels in both cell lines. Despite this, no differences in glycosylation were detected between monolayer cells and PIs. In contrast, although integrin $\alpha 6$ was expressed at much lower levels than $\alpha 3$, this integrin chain did show alternative glycosylation in PIs versus monolayer cells. Altogether, the facts that integrin $\beta 1$ can pair with several different integrin $\alpha$ chains and that integrin $\beta 1$ undergoes alternative glycosylation in PIs suggest that this integrin chain may be key in controlling the various integrin $\beta$-$\alpha$ associations in $\beta$ cells. These associations may improve with the glycosylation and maturation of the integrin $\beta 1$ protein.

The induction of PIs leads to three major changes when compared with monolayer cells. First, although monolayer cells
Integrin β1 Maturation in the Islet

showed punctuated staining of integrin β1 at the point of contact between the β cells and the culture surface, PIs exhibited a more dispersed and intense distribution of the receptor. Second, PI formation resulted in a higher percentage of integrin β1-expressing β cells and increased expression of cell surface protein relative to monolayers. Third, although monolayer cells showed only one size of integrin β1, PIs exhibited enhanced protein glycosylation similar to that of the native pancreas and primary hlslets. To the best of our knowledge, differential glycosylation of integrin receptors in general, and integrin β1 specifically, has not been documented in β cells. Previous reports in other cell types suggest that the highly glycosylated integrin β1 protein represents the mature form of the receptor, whereas the lower glycosylated protein is associated with reduced cell surface expression and is biologically inactive (31). For example, low molecular weight integrin β1 expression in both mature and immature keratinocytes led to reduced cell surface expression of the receptor, which was associated with altered culture conditions (30). Our results in β cells support these findings. Increased glycosylation and maturation of integrin β1 in PIs was coupled with an overall increase in the percentage of integrin β1-positive cells as well as an increase in the levels of integrin β1 cell surface expression. This increase was not associated with a total increase in integrin β1 protein levels.

Integrin β1 is important in mediating normal β-cell function. In vivo studies using knock-out mice lacking β-cell-derived integrin β1 showed that integrin β1 is required for β-cell expansion in the pancreas (35). In vitro blocking of integrin β1 has been shown to interfere with insulin release (24, 25, 36). Here we show a differential effect of integrin β1 blocking that is dependent upon the duration of antibody treatment. Short term exposure to anti-integrin β1 antibody did not affect insulin release in response to high glucose but did increase baseline secretion. Long term exposure resulted in a dramatic change in β-cell morphology and a reduction in insulin gene expression together with decreased Pdx1 and MafA mRNA levels. This reduction was associated with diminished insulin secretion in response to glucose stimulation, suggesting β-cell dedifferentiation. Further studies will be required to determine the effect of integrin β1 disruption.

Because integrin β1 glycosylation can be induced by the co-culturing of insulinoma cells and iECs, it is possible that under conditions of cellular stress, such as islet structure disruption and/or prolonged cell culture, integrin glycosylation may be reduced. Our results using murine PIs and primary hlslets suggest that islet structure and the presence of iECs are critical for the maintenance of integrin β1 glycosylation in β cells. For example, the mere removal of PIs from MS1 co-cultures as well as PI reattachment and formation of monolayers partially reversed the integrin β1 glycosylation in both insulinoma lines. Interestingly, integrin β1 glycosylation did not revert to monolayer levels, suggesting that some modification may prove more permanent in these cells. Similarly, prolonged culturing of primary human islets resulted in a marked reduction in the expression of the high molecular weight (M, 140) integrin β1in the complete islets. This change may be due to a gradual loss of iEC numbers/functionality overtime after islet purification, as suggested elsewhere (37, 38). The dynamic changes in integrin β1 glycosylation may allude to a previously unappreciated mechanism affecting the ability of β cells to respond to and interact with integrin binding ligands. Such an effect could reduce β-cell functionality. Future studies will be needed to examine the changes in integrin β1 glycosylation in β cells under conditions of inflammatory and metabolic stress.

In conclusion, we show here for the first time the ability of iECs to induce native glycosylation and maturation of the integrin β1 protein similar to that found in the native pancreas. This change is associated with de novo deposition of ECM proteins and leads to increased cell surface expression of the integrin receptor. Blocking of integrin β1 alters glucose sensitivity and insulin gene expression, whereas the disruption of islet structure can reverse the maturation of integrin β1. These dynamic changes in integrin β1 glycosylation may play an important role in altering β-cell function under pathological conditions.

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