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Cadherin-mediated adhesion in pancreatic islet cells is modulated by a cell surface *N*-acetylgalactosaminylphosphotransferase

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Summary

Rat pancreatic islet cells and RINm5F cells, an islet derived cell line, have at their cell surface an *N*-acetylgalactosaminylphosphotransferase (GalNAcPTase) similar to that found at the surface of chick neural retina cells and at the rat neuromuscular junction. On islet cells and RINm5F cells the GalNAcPTase is stably associated with cadherin cell-cell adhesion molecules. The effect of antibodies directed against the GalNAcPTase on homophilic, cadherin mediated adhesion was analyzed by measuring their effect on adhesion of islet and RINm5F cells to an immobilized anti-cadherin antibody. In this experimental paradigm anti-GalNAcPtase antibodies completely inhibit cadherin mediated adhesion. Furthermore, cadherin and GalNAcPTase co-distribute in islet and non-islet tissue. We conclude that pancreatic islet cell-cell adhesion is cadherin mediated and under the control of a tightly associated, cell surface GalNAcPTase.

Key words: cadherin, glycosyltransferase, pancreas, islet cells, adhesion.

Introduction

Using in vitro pancreatic islet cell aggregation systems, it has been demonstrated that structures closely resembling adult islets are generated from dispersed adult islet cells, suggesting that factors mediating cell-cell adhesion and morphogenesis persist in the terminally differentiated islet organ (Ono et al., 1979; Scharp et al., 1980; Montesano et al., 1983; Hopcroft et al., 1985; Halban et al., 1987). The cadherin molecules are obvious candidates for mediating this process. Their role in mediating calcium dependent cell-cell adhesion and their temporal and spatial distribution indicate that they play just such a role in vertebrate morphogenesis (Takeichi, 1990). Indeed, in the rat islet uvomorulin or E cadherin has been shown to mediate both B and non-B islet cell adhesion (Rouiller et al., 1991). What apparently distinguishes B and non-B adhesion is the presence of high levels of a calcium-independent adhesive system on non-B cells (Rouiller et al., 1991).

Cadherin mediated adhesion involves interactions with the cytoskeleton as well as with extracellular components. Removing or altering the C-terminal cytoplasmic domain of cadherin abolishes its ability to form adhesions, even though the molecule is expressed at the cell surface (Nagafuchi and Takeichi, 1988; Kintner, 1992). Furthermore, in the chick retina N-cadherin is tightly associated with a cell surface *N*-acetylgalactosaminylphosphotransferase (GalNAcPTase; Balsamo and Lilien, 1990) which modulates its association with the cytoskeleton (Balsamo et al., 1991). Antibodies directed against the enzyme completely block N-cadherin mediated adhesion and result in uncoupling of cadherin from its association with the cytoskeleton (Balsamo et al., 1991). The GalNAcPTase may have a broader role in modulating adhesion. This is suggested by the ability of anti-GalNAcPTase antibodies to inhibit neurite outgrowth mediated by receptors in at least three distinct families (Gaya-Gonzalez et al., 1991): cadherin, G4 (a member of the immunoglobulin family; Lagenaur and Lemmon, 1987) and integrin.

We have observed the GalNAcPTase not only in retina but in many other tissues (Scott et al., 1990 and unpublished). The purpose of the present study was to determine if the GalNAcPTase is present in pancreatic islet tissue, and if so, whether it is associated with, and modulates cadherin mediated adhesion.

Materials and methods

Antibodies

C1 is a rabbit polyclonal antibody against a highly conserved 20 amino acid sequence at the carboxy terminus of N-, E- and P-cadherin as well as chicken L-CAM (compared in Hatta et al., 1988). The actual sequence used was from L-CAM (ENLKAADTDPTAPPYDVFDYEG; underline indicates conserved residues). Antibody preparation and affinity purification were as described (Harlow and Lane, 1988). RR2 is a rabbit polyclonal antibody that recognizes N-cadherin and inhibits cell-cell adhesion of embryonic chick neural retina cells in vitro (Crittenden

et al., 1987). It also recognizes a 130 kDa molecule in various embryonic tissues including chick skin, heart, lung, skeletal muscle, liver and kidney (Crittenden et al., 1987). RR1 is a rabbit polyclonal antibody specific for N-cadherin (Crittenden et al., 1987). 1B11 and 7A2 are mouse monoclonal IgM antibodies which recognize cell surface GalNAcPTase in chick neural retina (Balsamo et al., 1991) and vertebrate skeletal muscle (Scott et al., 1990). 1B11 inhibits N-cadherin mediated neurite outgrowth (Gaya-Gonzalez et al., 1991) and cell-cell adhesion in vitro (Balsamo et al., 1991). In contrast to 1B11, 7A2 inhibits neither of these N-cadherin mediated activities, indicating that it reacts with a different epitope than 1B11 (Balsamo et al., 1991; Gaya-Gonzalez et al., 1991).

Tissue and cell preparation

Chick tissues were obtained from 10-day embryos. Rat tissues were obtained from 250 g Holtzman rats. Islets of Langerhans were prepared by collagenase digestion of pancreas as described previously (Gotoh et al., 1987), and cultured for one to two days before use to eliminate contamination by acinar cells and ductal cells. Islet tissue was dispersed into single cells using trypsin and mechanical shear (Pipeleers and Pipeleers-Marichal, 1981). The resulting cells were allowed to recover for 3 h in culture before use. RINm5F cells are a subline (Oie et al., 1983) of an X-radiation-derived, insulin-secreting cell line (Chick et al., 1977) and were a gift of Dr Orion Hegre.

Western blots

Tissues were homogenized in hot 2 × SDS sample buffer (6% sodium dodecyl sulphate, 10% 2-mercaptoethanol, 20% glycerol and Bromophenol blue in 0.25 M Tris, pH 6.8), boiled for 2 min and the supernatant loaded onto a 7% polyacrylamide gel. Alternatively, tissues were homogenized and incubated for 30 min in Tris-buffered saline (TBS) containing 25 μg/ml each of antipain, leupeptin, chymostatin and pepstatin, 15 mM 1,10-phenanthroline and 100 μg/ml DNAase (TBSTI), then centrifuged at 10,000 ***g*** for 1 min to pellet nuclei and cytoskeleton. The resulting supernatant was mixed 1:1 with 2 × SDS sample buffer, boiled for 1 min and electrophoresed. After SDS-PAGE, the proteins were transferred to Immobilon (Millipore) and incubated overnight at 4°C in TBS containing 5% nonfat dry milk. Membranes were washed in TBS-0.5% Tween 20 (TBSTw), incubated for 3 hours at room temperature with primary antibodies in TBSTw, and washed in TBSTw. They then were incubated for 1 hour at room temperature with alkaline phosphatase or horseradish peroxidase-conjugated secondary antibodies. Reactive bands were detected using NBT/BCIP or the ECL system (Amersham).

Immunoprecipitation

To determine if cadherin and the GalNAcPTase were non-covalently associated, pancreas islet tissue or RINm5F cells were lysed in TBSTI and incubated with anti-GalNAcPTase antibody 1B11 or control IgM at 4°C. Immunobeads coated with goat anti-mouse IgM were then used to pellet antigen-antibody complexes. Pellets were resuspended in SDS sample buffer, separated by SDS-PAGE, transferred to Immobilon (Millipore) and reacted with anti-cadherin antibody as described above.

To further demonstrate that the cadherin/GalNAcPTase complex was at the cell surface, RINm5F cell surface polypeptides were labeled prior to immunoprecipitation with 1B11. Intact cell layers were incubated with 0.5 mg/ml NHS-LC-biotin (Pierce) in PBS at 4°C for 30 min. The labeled cells were washed and homogenized in TBSTI. To disrupt non-covalent associations, one aliquot was made 1% in SDS, boiled and diluted to 0.1% SDS with TBSTI prior to immunoprecipitation. The immunoprecipitates were separated by SDS-PAGE, transfered to Immobilon (Millipore) and visualized with alkaline phosphatase-conjugated avidin.

Cell-cell adhesion assay

The binding of radiolabeled RINm5F cells to monolayers of unlabeled RINm5F cells was assayed as previously described (Balsamo et al., 1991). Briefly, RINm5F cells obtained directly from culture flasks (i.e. target cells) or after overnight incubation in culture medium supplemented with ^{14}C-labeled amino acid mixture (i.e. probe cells) were harvested by trypsin treatment and allowed to recover from the isolation procedure by 3 hours of incubation at 37°C in culture medium. Suspensions of single target cells were centrifuged onto poly-L-lysine coated wells in 96-well culture plates. The resulting cell monolayers were treated with a solution of poly-L-glutamate just before the assay, to prevent binding of probe cells to exposed poly-L-lysine. Suspensions of single probe cells in PBSCM (phosphate buffered saline with 1 mM Ca^{2+},1 mM Mg^{+}, pH 7.8) were added at 5×10^4 to 7.5×10^4 cells/well. Additions of 10 mM EDTA in PBSCM (pH 7.8) or various antibodies at 1 mg/ml (diluted in PBSCM) were made to the probe cells, which were maintained at 4°C for 30 min. Probe cell suspensions were added to the monolayer of target cells and incubated for 30 min at 37°C. Unbound cells were aspirated to counting vials. Bound cells were washed, resuspended and transferred to counting vials. Radioactivity in free and bound cells was determined by liquid scintillation spectrometry.

Cell-substratum adhesion assay

Adhesion to immobilized antibodies was performed as previously described (Balsamo et al., 1991). 96-well plates were incubated overnight at 4°C with affinity-purified anti-cadherin (RR2 IgG) or anti-GalNAcPTase (1B11 IgM) antibodies at 0.1 mg/ml diluted in PBSCM. The wells then were washed in assay buffer (PBSCM) and blocked with 1% BSA for 1 h at room temperature.

Adhesion of cells to immobilized antibodies was assayed essentially as above. Labeled islet cells were prepared from tissues incubated overnight in medium containing ^{14}C-labelled amino acid mixture. Radiolabeled RINm5F (10^4 per well) or islet cells (7.5×10^4 per well) were incubated with anti-GalNAcPTase antibody or control IgM (at 0.1 mg/ml each) for 30 min at 4°C prior to addition to coated wells.

Histochemistry

Adult rat pancreas was fixed in 1% paraformaldehyde overnight at room temperature, quick frozen and sectioned by cryostat; 15 μm sections were affixed to poly-L-lysine-coated glass slides and preincubated at room temperature with 10% goat serum in PBS for 15 min and in 3% hydrogen peroxide for 15 min. For localization of antigens, sections were washed with 0.05% Tween 20 in PBS and incubated overnight with affinity-purified primary antibodies: 1B11, a mouse monoclonal IgM anti-GalNAcPTase (see above); C1, a rabbit polyclonal anti-cadherin IgG (see above); or suitable IgM or IgG controls. To reduce non-specific binding of IgM, pancreatic sections were pretreated with 1% bovine IgM in PBS for 2 h at room temperature. FITC-conjugated secondary antibodies were applied overnight at 4°C. After thorough washing in PBS, sections were examined by confocal microscopy.

Results

Cadherin and the cell surface N*-acetylgalactosaminyl-phosphotransferase (GalNAcPTase) are present and associated in pancreatic islet tissues*

A polyclonal antibody, raised against a carboxy-terminal sequence derived from L-CAM and highly conserved among cadherins, recognizes a polypeptide in a variety of rat tissues, including pancreas (Fig. 1 top, lane d) and iso-

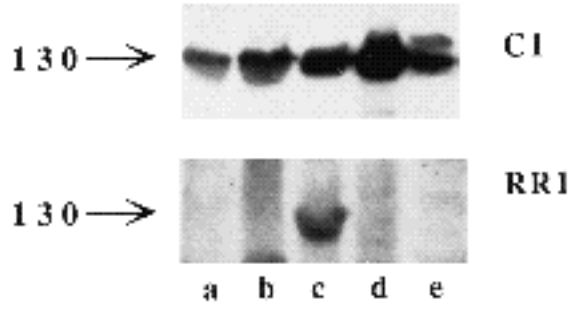

Fig. 1. Reactivity of anti-cadherin antibodies C1 and RR1 with rat cells and tissues. Lane (a) RINm5F cells; (b) rat islet tissue; (c) rat brain tissue; (d) rat pancreas tissue; (e) rat liver tissue.

lated islet cells (Fig. 1 top, lane b) at the same molecular mass as that found in chick brain (Fig. 1 top, lane c). C1 also recognizes proteins of similar molecular mass (≈130 kDa) in RINm5F cells, a cell line derived from a rat insulinoma (Fig. 1 top, lane a). RR1, an antibody specific to N-cadherin (Crittenden et al., 1987) recognizes only the rat brain cadherin (Fig. 1 bottom, lane c), indicating that the C1 reactive species in rat pancreas and in RINm5F cells is not N-cadherin. This is consistent with the recent report that E-cadherin is the cadherin species present in rat pancreas (Rouiller et al., 1991).

Antibodies to the chick retina GalNAcPTase recognize a polypeptide in pancreas (Fig. 2 top, lane a), isolated islets (Fig. 2 top, lane c) and RINm5F cells (Fig. 2 top, lane b)

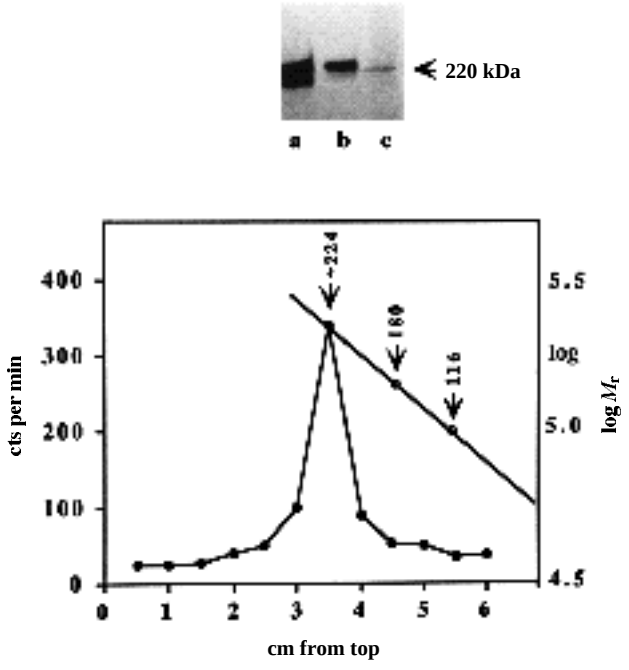

Fig. 2. Coincidence of anti-GalNAcPTase antibody reactivity with GalNAcPTase activity. Top: immunoblots using anti-GalNAcPTase antibody on rat pancreas (lane a), RINm5F (lane b) and rat islet tissue (lane c). Bottom: GalNAcPTase activity assayed using 0.5cm pieces of a strip of nitrocellulose containing rat islet tissue run in parallel to the above immunoblots.

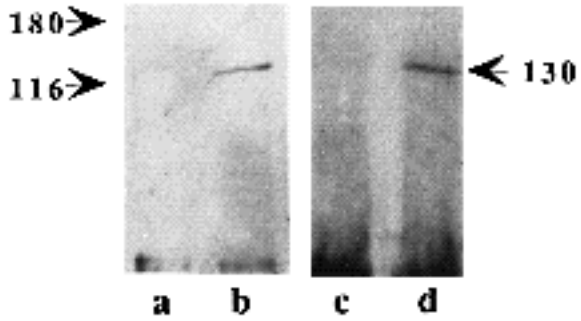

Fig. 3. Co-immunoprecipitation of cadherin and GalNAcPTase in islet tissue and RINm5F cells. Homogenates of islet tissue (lanes a and b) or RINm5F cells (lanes c and d) were immunoprecipitated with anti-GalNAcPTase antibody (lanes b and d) or control IgM (lanes a and c), the precipitates separated by SDS-PAGE, transferred to nitrocellulose and reacted with anti-cadherin antibody.

of identical molecular mass to that found in chick retina, ~220 kDa. Furthermore, when assayed for transferase activity, the isolated band from islet tissue incorporates radiolabeled sugar from nucleotide sugar into an exogenous acceptor (Fig. 2 bottom).

Coimmunoprecipitation experiments indicate that the two molecules, cadherin and GalNAcPTase, are associated in islet tissue. Anti-GalNAcPTase antibody 1B11 was used to precipitate cadherin from neutral detergent extracts, the precipitates were separated by SDS-PAGE and immunoblotted with anti-cadherin antibody C1. Cadherin is present in the precipitates prepared with anti-GalNAcPTase antibody but not with control IgM (Fig. 3, lanes a and b).

Since RINm5F cells are derived from islet tissue, we also determined if cadherin and the GalNAcPTase are associated in these cells. Again, neutral detergent extracts were immunoprecipitated with the anti-GalNAcPTase antibody 1B11, the precipitates separated by SDS-PAGE and immunoblotted with anti-cadherin antibody C1 (Fig. 3, lanes c and d). As in islet tissue, the two molecules are physically associated as evidenced by the presence of cadherin in the anti-GalNAcPTase precipitates (Fig. 3).

To determine if the GalNAcPTase is at the outer cell surface, intact RINm5F cells were labeled with biotin and neutral detergent extracts precipitated with anti-GalNAcPTase antibody 1B11; two labeled molecules are found in the precipitates following separation by SDS-PAGE and staining with alkaline phosphatase-conjugated avidin. One comigrates with cadherin and the other with the GalNAcPTase (Fig. 4). When neutral detergent extracts are treated with SDS prior to immunoprecipitation with 1B11, only labeled GalNAcPTase is present (Fig. 4).

Adhesion of islet cells is cadherin mediated and modulated by the GalNAcPTase

Since RINm5F cells are derived from islet tissue and cadherin and GalNAcPTase are associated in RINm5F as in islet tissue, we used these cells as a model to study the role of the GalNAcPTase in adhesion. Labeled RINm5F "probe" cells were added to monolayers of RINm5F cells and calcium-dependent adhesion was measured as the difference

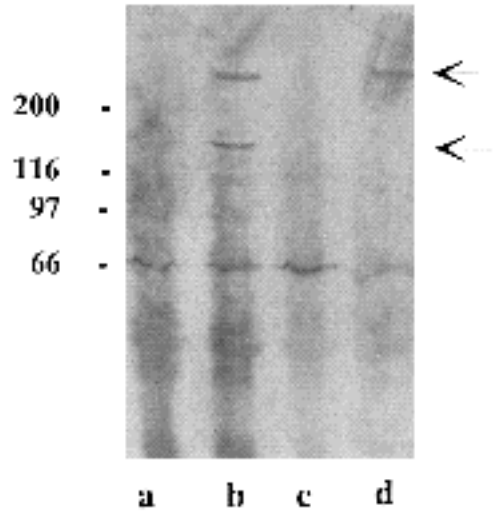

Fig. 4. Localization of the GalNAcPTase at the outer cell surface. Intact RINm5F cells were labeled with biotin and homogenates precipitated with anti-GalNAcPTase antibody (lanes b and d) or control IgG (lanes a and c), separated by SDS-PAGE and reacted with alkaline phosphatase-conjugated avidin. When homogenates are prepared in neutral detergent (lanes a and b) both cadherin and GalNAcPTase are seen (arrows). When neutral detergent homogenates are treated with SDS prior to immunoprecipitation (lanes c and d) only the GalNAcPTase is present (upper arrow).

between control assays and assays carried out in the presence of EDTA (10 mM). EDTA results in approximately 62% inhibition of cell-cell binding (Fig. 5). Anti-cadherin antibody RR2 results in 55% inhibition, indicating that most, if not all, of the calcium-dependent adhesion is mediated by cadherins. Addition of anti-GalNAcPTase antibody

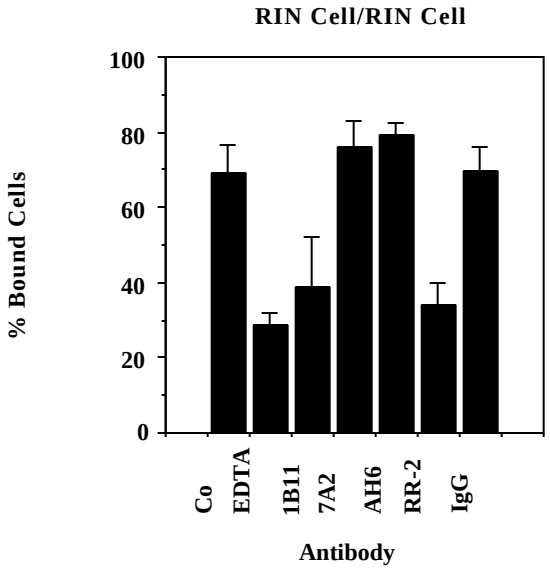

Fig. 5. Adhesion of labeled RINm5F cells to monolayers of RINm5F cells under control conditions (Co), in the presence of EDTA and in the presence of anti-cadherin (RR-2), anti-GalNAcPTase (1B11, 7A2) and control (AH6 and IgG) antibodies.

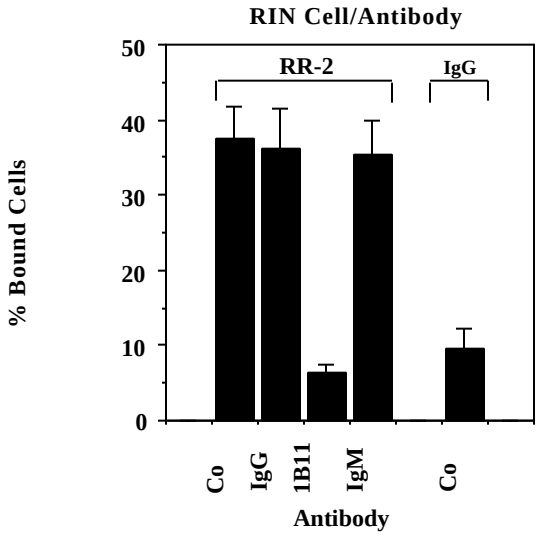

Fig. 6. Adhesion of labeled RINm5F cells to surfaces coated with anti-cadherin antibody RR-2 or control IgG under control conditions (Co), in the presence of anti-GalNAcPTase antibody (1B11), and in the presence of control IgG and IgM.

1B11 results in approximately 50% inhibition, while 7A2, an anti-GalNAcPTase antibody which binds to the cell surface but is not inhibitory (Balsamo et al., 1991), is without effect on binding.

To determine if the effect of anti-GalNAcPTase antibody 1B11 on adhesion of RINm5F cells is on cadherin mediated adhesion, anti-cadherin antibody RR2 was applied to wells as a substrate for cell binding. In this paradigm anti-GalNAcPTase antibody 1B11 inhibits approximately 80% of cadherin mediated adhesion (Fig. 6).

To insure that the properties of the RINm5F cell line reflect those of islet tissue, freshly dispersed islet cells were assayed for their ability to adhere to anti-cadherin antibody RR2 and for the effect of anti-GalNAcPTase antibody 1B11. As with RINm5F cells, 1B11 is a potent inhibitor of cadherin mediated adhesion among islet cells (Fig. 7).

Cadherin and GalNAcPTase co-distribute in islet and acinar tissue

Adult rat pancreas was stained with the anti-cadherin antibody C1 and visualized with FITC-conjugated goat anti-rabbit IgG. A fine band of fluorescence was detected at basal and lateral margins of acinar cells (Fig. 8A and B). In addition, diffuse cytoplasmic staining was seen in some acinar cells (Fig. 8A and B). In contrast, islet cells generally exhibited diffuse cytoplasmic staining with C1, extending to the islet-acinar boundary (Fig. 8A and B), suggesting that cadherin molecules are present in all islet endocrine cell types.

To localize the GalNAcPTAse, sections of adult rat pancreas were stained with the anti-GalNAcPTase antibody 1B11 and visualized with FITC-goat anti-mouse Ig. The distribution of GalNAcPTase is very similar to that seen with anti-cadherin antibody C1; staining is at the margins of both acinar and islet endocrine cells and is diffuse within the islet (Fig. 8C). The most obvious difference in staining

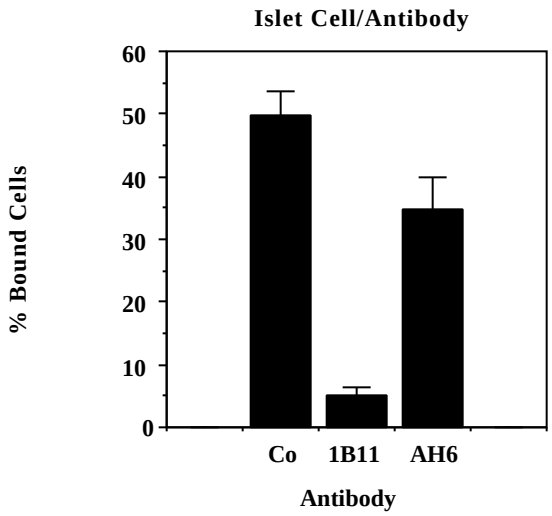

Fig. 7. Adhesion of labeled islet cells to a surface coated with anti-cadherin antibody RR-2 under control conditions (Co), in the presence of anti-GalNAcPTase antibody (1B11) and in the presence of control IgM antibody (AH6).

with anti-cadherin versus anti-GalNAcPTase antibodies is the texture of the staining; cadherin is punctate, both in the cytoplasm and at the surface, while GalNAcPTase is smoothly distributed (compare Fig. 8A and C).

Discussion

Cadherin mediated adhesion among islet cells is modulated by a cell surface N-acetylgalactosaminylphosphotransferase (GalNAcPTase). Perturbation of the transferase with some, but not all, antibodies specific to the GalNAcPTase results in an inhibition of homophilic cadherin mediated adhesion. To insure that anti-GalNAcPTase antibodies were inhibiting homophilic cadherin adhesion, we assayed the effect of anti-GalNAcPTase antibodies on the adhesion of cells to anti-cadherin antibodies. The resulting inhibition indicates that the ability of cadherin to form a stable bond, whether to another cadherin molecule or to an antibody, is affected by its interaction with the GalNAcPTase.

We used RINm5F cells as a model to initiate our studies on islet cell adhesion properties (see also Rouiller et al.,

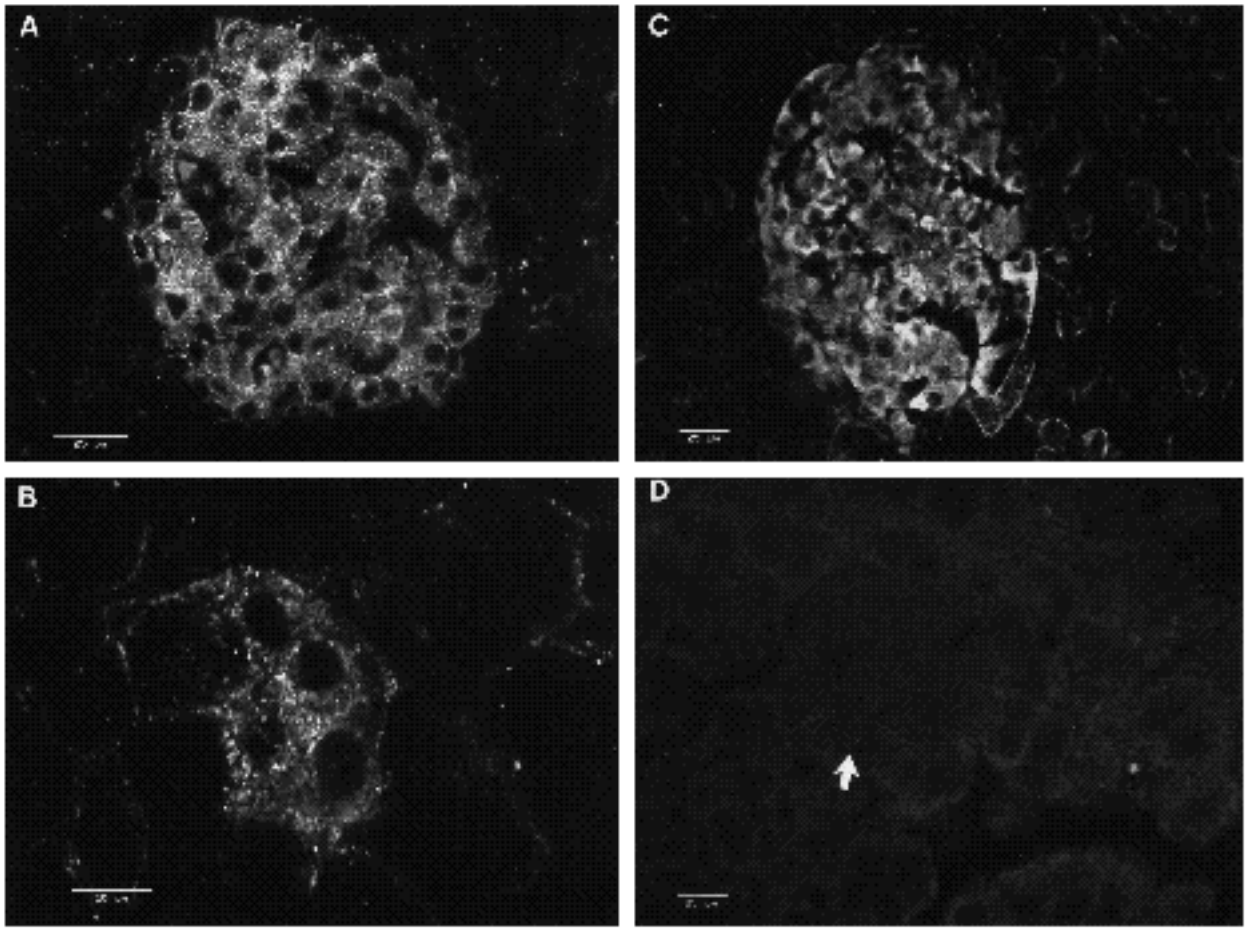

Fig. 8. Immunohistochemical localization of cadherin and GalNAcPTase in rat pancreas. Frozen sections of paraformaldehyde-fixed adult pancreas were reacted with anti-cadherin antibody (A and B) and with anti-GalNAcPTase antibody (C) or control IgM (D) followed by flourescent secondary antibody. Cadherin is distributed diffusely in the cytoplasm and at the cell margins of islets or small clusters of islet cells (A and B). In contrast, acinar cells have a fine band of fluorescence at the basal and lateral margins (B). The distribution of the GalNAcPTase is similar to cadherin; however, staining is smooth as opposed to the punctate distribution seen with anti-cadherin antibody (C). Little or no fluorescence is seen with control IgM (D) in islets (arrow) or ascinar cells. Bars, 25 μm (A,C,D); 18 μm (B).

1990). RINm5F cells provide a readily available source of cultured islet B cells. As in other cultured islet cell lines, the phenotypic expression of certain B cell properties is, however, altered. For example, RINm5F cells exhibit characteristics that resemble immature B cells, such as inappropriate insulin secretory responses to glucose (Nielsen et al., 1985). Nevertheless, the cell line has been invaluable in studying various aspects of B cell physiology. In our in vitro adhesion assay, RINm5F cell binding to a RINm5F cell monolayer is inhibited by EDTA and anti-cadherin antibody to the same extent, indicating that all calcium-dependent adhesion is cadherin mediated. Furthermore, as in islet cells, cadherin mediated adhesion is modulated by anti-GaINAcPTase and cadherin and GalNAcPTase are associated in a complex stable to neutral detergent. These observations demonstrate that the RINm5F cells used in this study express the same calcium-dependent adhesive properties as those of islet cells and are a valid model for adhesion studies.

In islet and acinar cells, cadherin and GalNAcPTase co-distribute. Histochemistry reveals that islet cells have both cadherin and transferase at the cell surface and within the cytoplasm, while in acinar cells cadherin and GalNAcPTase are restricted to the cell surface. Thus, islet tissue is enriched in cadherin and GalNAcPTase relative to non-islet tissue (acinar).

Islet tissue is composed of endocrine B and non-B cells which sort during reaggregation in vitro (Ono et al., 1979; Scharp et al., 1980; Montesano et al., 1983; Hopcroft et al., 1985). It has been suggested that sorting among these two islet cell types is due to calcium-independent adhesion molecules (Rouiller et al., 1991). Flow cytometric measurements indicate that there is no difference in the cell surface concentration of cadherin (Rouiller et al., 1991), while the level of N-CAM (i.e. calcium-independent adhesion) is much higher on non-B than on B cells (Rouiller et al., 1990).

During development, both calcium-dependent and calcium-independent CAMs are located at interfaces of sorting or separating tissue masses (Takeichi, 1990; Edelman and Crossin, 1991) and, all other things being equal, different CAMs can mediate sorting (Nose et al., 1988; Friedlander et al., 1989). The GalNAcPTase is tightly associated with, and modulates the activity of, two different cadherins, N and E, in the retina (Balsamo and Lilien, 1990) and pancreas, respectively, and modulates the activity of members of two additional CAM families, the immunoglobulin/N-CAMs and integrins (Gaya-Gonzales et al., 1991). In the chick retina, modulation of cadherin mediated adhesion by the GalNAcPTase is accompanied by the disruption of the cadherin-actin association (Balsamo et al., 1991), suggesting that the GalNAcPTase exerts its effect on cell-cell or cell-substratum binding through perturbation of the interaction of CAMs with the cytosekeleton. Perturbation of the interaction of cadherin with the cytoskeleton by other means also inhibits cadherin mediated adhesion (Nagafuchi and Takeichi, 1988; Kintner, 1992) and alteration of the cytoplasmic domain of CAMs can alter the pattern of cell sorting (Jaffe et al., 1990). By altering the association of CAMs with the cytoskeleton, the GalNAcPTase may affect the ability of cells to sort from each other during development and during experimental reconstruction of tissues in vitro.

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