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Expression of neural cell adhesion molecule (N-CAM) in rat islets and its role in islet cell type segregation

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SUMMARY

Endocrine cell types are non-randomly distributed within pancreatic islets of Langerhans. In the rat, insulin-secreting B-cells occupy the core of the islets and are surrounded by A-, D- and PP-cells, secreting glucagon, somatostatin and pancreatic polypeptide, respectively. Furthermore, dissociated islet cells have the ability in vitro to form aggregates with the same cell-type organization as native islets (pseudoislets). These observations suggest that a differential expression of cell adhesion molecules (CAMs) might characterize B- and non-B-cells (A-, D- and PP-cells), and be in part responsible for the establishment and maintenance of islet architecture. Indirect immunofluorescence using antibodies against CAMs and islet hormones was performed on serial sections of the splenic and duodenal parts of the rat pancreas. Staining for the Ca^{2+}-dependent CAM E-cadherin was detected on both exocrine and endocrine tissue and was uniform over the entire islet section, in both pancreatic regions. By contrast, staining for the Ca^{2+}-independent neural CAM (N-CAM) was restricted to endocrine tissue and nerve endings. Furthermore, N-CAM staining of endocrine cells was stronger in the islet periphery, a region composed mostly of non-B-cells. Serial sections demonstrate that cells staining strongly for N-CAM in the splenic part correspond to glucagon cells and in the duodenal part to pancreatic polypeptide cells. Within pseudoislets in vitro a stronger staining for N-CAM was also observed on peripheral cells, corresponding to non-B-cells.These data suggest that a difference in the expression of N-CAM might play a role in the segregation between islet cells. To verify this hypothesis, isolated islet cells were allowed to form aggregates in culture, in the presence of non-immune or anti-N-CAM Fab fragments. Inhibition of N-CAM function by specific Fab fragments prevented the segregation between cell types that normally occurs within 5 days. In conclusion, higher levels of N-CAM characterize peripheral islets cells, irrespective of their secretory type, and anti-N-CAM Fab fragments prevent islet cell type segregation within pseudoislets in vitro. These results suggest that differences in N-CAM expression between cell types contribute to the characteristic distribution of cells within islets of Langerhans.

Key words: N-CAM, cell adhesion, islets of Langerhans

INTRODUCTION

Islets of Langerhans display a characteristic cell-type organization. In the rat they are formed as a core of insulin-secreting B-cells, surrounded by the other endocrine cell types (‘non-B-cells’, secreting glucagon, somatostatin and pancreatic polypeptide) (Orci and Unger, 1975). This well defined architecture is highly perturbed in animal models of diabetes (Baetens et al., 1978; Gomez Dumm et al., 1990; Orci et al., 1976b; Unger et al., 1978; Starich et al., 1991; Unger and Orci, 1981). Exploring the molecular basis of islet cell-type organization might result in a better understanding of its role in islet function.

Extensive immunofluorescence studies have revealed a dynamic modulation of expression of certain cell adhesion molecules (CAMs) during morphogenesis and histogenesis (Edelman, 1986). The expression pattern of these molecules correlates with the positional segregation of cell layers within epithelia (Hatta and Takeichi, 1986; Nose and Takeichi, 1986; Rutishauser, 1984; Thiery et al., 1982). E-cadherin (epithelial-cadherin), a widely represented Ca^{2+}-dependent CAM (Yoshida-Noro, 1984), homologous to chicken LCAM (Gallin et al., 1983) and mouse uvomorulin (Hyafil et al., 1980, 1981), is found on most epithelia and has been shown to be responsible for the Ca^{2+}-dependent adhesion of islet cells (Rouiller et al., 1991; Bauer et al., 1992). Neural CAM (N-CAM) is the best studied Ca^{2+}-independent CAM, which was first described in the nervous system. The expression of N-CAM is developmentally regulated in a variety of tissues, derived from all three germ layers (Rutishauser, 1984). The presence of N-CAM and E-cadherin immunoreactivity on adult islet cells in situ has been reported in rat (Langley et al., 1989; Rouiller et al., 1990, 1991), mouse (Begemann et al., 1990) and man (Eidelman et al., 1989).

Transfection studies have demonstrated that qualitative and/or quantitative differences in CAM expression result in segregation between clones of cells in culture (Nose et al., 1988; Friedlander et al., 1989). The pioneer studies of Holtfreter (1939) have shown that mixed suspensions of dispersed embryonic cells from various tissues re-establish normal histotypic relationships when allowed to reaggregate in culture. This ability to reorganize in vitro has been demonstrated for both neonatal (Montesano et al., 1983) or adult (Halban et al., 1987; Hopcroft et al., 1985; Tze and Tai, 1982) rat islet cells, providing a good model with which to characterize the molecular basis of islet histogenesis. In the present study, specific antibodies have been used to analyse the distribution of E-cadherin and N-CAM within islets in situ, and pseudoislets in vitro, and to evaluate the contribution of N-CAM in the normal segregation between B and non-B-cells within pseudoislets. The results strongly support a role for N-CAM in the establishment and maintenance of islet architecture.

MATERIALS AND METHODS

CAM localization by indirect immunofluorescence

For in situ studies, adult Sprague-Dawley rats were anaesthetized and perfused through the left ventricle with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Both the duodenal and splenic parts of the pancreas were dissected out and post-fixed for 6 hours in the same fixative. After rinsing overnight in 0.1 M phosphate buffer, the tissue was kept in 0.9% NaCl supplemented with 15% sucrose. Pieces of both parts of the pancreas were frozen and mounted on microtome chucks. For aggregates in vitro (pseudoislets), separate populations of B- and non B-cells were obtained by autofluorescence-activated cell sorting (see below), mixed at a ratio of 60% non-B-cells and 40% B-cells, to account for the smaller size of non-B-cells, and resuspended at a concentration of 2×10^6 cells/ml in DMEM containing 5 mM glucose, 10% FCS, 10 mM HEPES. After 5 days in culture in a humidified atmosphere of 95% air and 5% CO_2 at 37°C, several aggregates had formed. These pseudoislets were fixed (6-hour paraformaldehyde incubation) and prepared for cryomicrotomy as described for the pancreata.

After permeabilization, consecutive serial sections, 8 μm thick, were incubated overnight at 4°C with the following antibodies: anti-N-CAM antibodies (from Dr U. Rutishauser, 1:1000; or from Dr C. Goridis, 1:1500), anti-E-cadherin (anti-mouse uvomorulin, Dr R. Kemler, 1:200), anti-glucagon (Dr R. Unger, 1:1000), anti-pancreatic polypeptide (Dr R. Chance, 1:1000), anti-somatostatin (Dr R. Benoit, 1:2000) and anti-insulin (Dr P. Wright, 1:1000). After rinsing with phosphate-buffered saline, FITC-conjugated goat anti-rabbit or sheep anti-guinea pig IgG diluted 1:200 and 1:400, respectively, were used as second antibodies for 1 hour at room temperature. Finally, sections were counterstained with Evan's blue and viewed in an Axiophot fluorescence microscope. Control sections were incubated in parallel, omitting the first antibody.

Preparation of islet cells

Rat pancreatic islets were isolated by a modification of the method of Sutton et al. (1986), as previously described (Rouiller et al., 1990). The yield was about 800 islets/rat. Islets were then washed twice with Mg^{2+}-, Ca^{2+}-free phosphate buffered saline containing 0.5 mM EDTA and resuspended in 1.5 ml of Puck's buffer containing 0.16 mg/ml of trypsin (activity against casein, 1:250) and 0.1 mM EDTA. Digestion was carried out for 6-7 minutes at 37°C, until only few doublet cells remained. The reaction was ended by adding 10 ml ice-cold Krebs-Ringer-bicarbonate (KRB) buffer, pH 7.4, containing 0.5% BSA, 2.5 mM glucose, and 10 mM HEPES. Following centrifugation for 8 minutes at 4°C and 600 ***g,*** cells were taken up in the same buffer to a final concentration of 3×10^6 cells/ml. The yield was 1×10^6 to 1.5×10^6 cells/pancreas.

Autofluorescence-activated cell sorting of islet B- and non-B-cells

Dispersed islet cells were analysed in either an Epics-V flow cytometer connected to an MDAS microcomputer (Coulter Electronics, Hialeah, FL), or a FACStar-Plus (Becton-Dickinson, Erembodegem, Belgium). Cellular autofluorescence, excited by an argon laser beam tuned to 488 nm at 500-600 mW output power, was plotted against forward light scatter (FLS), which is related to cellular size (Loken and Herzenberg, 1975). As reported (Van De Winkel et al., 1982), at 2.5 mM glucose two islet cell populations became apparent when particle flavin adenine dinucleotide (FAD) autofluorescence (510-550 nm) was plotted against light scatter. Sorting 'windows' were then externally applied around both populations in order to deflect viable cells into one or the other collecting tube containing sterile KRB-BSA.

The distribution of insulin- and glucagon-containing cells was assessed by indirect immunofluorescence on cells attached on poly-L-lysine-coated cover-glasses. This revealed that one sorted population contains 93±1.6% non-B-cells, while the other population contains 95±0.7% B-cells (Rouiller et al., 1990). About 1×10^6 non-B-cells and 2.8×10^6 B-cells are obtained from 6 rats.

Colorimetric pseudoislet segregation assay and Fab perturbation studies

Islet cell type segregation within pseudoislets in vitro was studied using a modification of the method of Friedlander et al. (1989), as previously described (Rouiller et al., 1991). Briefly, following sorting, islet B- and non-B-cells were incubated for a period of 18-20 hours at a dilution of 3×10^4 cells/ml in DMEM containing 5 mM glucose, 5% FCS, in the presence of either 3 mg/ml of the vital fluorescent dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indo-carbocyanine perchlorate (DiI) (B-cells) or 10 mg/ml of 3,3′-dioctadecyl-oxa-carbocyanine perchlorate (DiO) (Molecular Probes, Eugene, OR) (non-B-cells). DiI is maximally excited by green light and fluoresces red-orange when viewed through rhodamine filters, while DiO is excited in the blue and fluoresces green when observed through fluorescein filters.

After washing, the two differentially labeled cell populations were mixed together at a ratio of 60% non-B-cells to 40% B-cells, and resuspended at a concentration of 2×10^6 cells/ml in DMEM containing 5 mM glucose, 10% FCS, 10 mM HEPES, and either 500 μg/ml of polyclonal rabbit anti-N-CAM Fab fragments or non-immune Fab fragments. On occasion, Fab fragments of non-CAM-related anti-islet IgGs were used, giving essentially the same results as non-immune Fab fragments. Cells were then placed in 35 mm × 10 mm non-adherent Petri dishes as microdroplets of 40 μl each, and maintained in a humidified atmosphere of 95% air and 5% CO_2 at 37°C for 5 days. Each microdroplet was supplemented daily with 30 μg of the appropriate Fab fragment.

Cell type segregation and organization within pseudoislets were monitored at day 5 by laser fluorescence microscopy using a confocal imaging system (Bio-Rad MRC-600, Microscience Division, Watford, England), as previously described (Rouiller et al., 1991). The partial overlap existing between the emission spectra of the two carbocyanines allowed us simultaneously to identify on the same scanned plane both DiO and DiI fluorescence as different grey levels. Clusters of pure non-B-cells labeled with the green fluorescent DiO, and clusters of pure B-cells labeled with the red fluorescent DiI were used as controls to identify the grey band-width corresponding to the green (DiO) or red (DiI) fluorescence, respectively. Confocal sections corresponding to the equatorial regions of each aggregate were chosen to identify consistently the organization of cells within the core of the

pseudoislets. Within each experiment, 20-30 pseudoislets were obtained and were all examined for cell type organization. For acquired images, DiO and DiI grey bands were attributed green and red pseudocolors, respectively. For statistical evaluation, the proportion of total green surface (non-B-cells) located in the pseudoislet mantle (outer third of the equatorial plan) was measured using an electronic pen and a graphic tablet (Tectronix type 4953) connected to a microprocessor system (IMSAI type 8080). The paired *t*-test was used for comparison between control pseudoislets and aggregates formed in the presence of anti-N-CAM Fab fragments.

RESULTS

Identification of pancreatic E-cadherin and N-CAM by indirect immunofluorescence

Immunofluorescence staining for E-cadherin was detected on the membrane of the acinar and ductal cells of the exocrine pancreas as well as on the endocrine cells within the islets. The staining pattern was uniform over the central and the peripheral regions of the islets occupied, respectively, by the B- and non-B-cells (Fig. 1). No difference was observed between islets of the splenic and duodenal parts of the pancreas. These results suggest that cell type segregation within islets is probably not mediated by differences in E-cadherin expression. As expected from previous studies (Moller et al., 1992; Rouiller et al., 1990), a completely different pattern of expression prevails for N-CAM. In the exocrine pancreas a bright immunofluorescence for N-CAM was found over nerve fibres and ganglia, whereas acinar and ductal tissues showed no labeling. In the islets, all endocrine cells showed a membranous N-CAM staining, which was stronger on cells located in the islet periphery than on cells occupying the core (Fig. 2, upper panel). Serial sections incubated with anti-insulin antibody demonstrate that the central cells that are weakly fluorescent for N-CAM correspond to the insulin-secreting B-cells (Fig. 2, lower panel). As

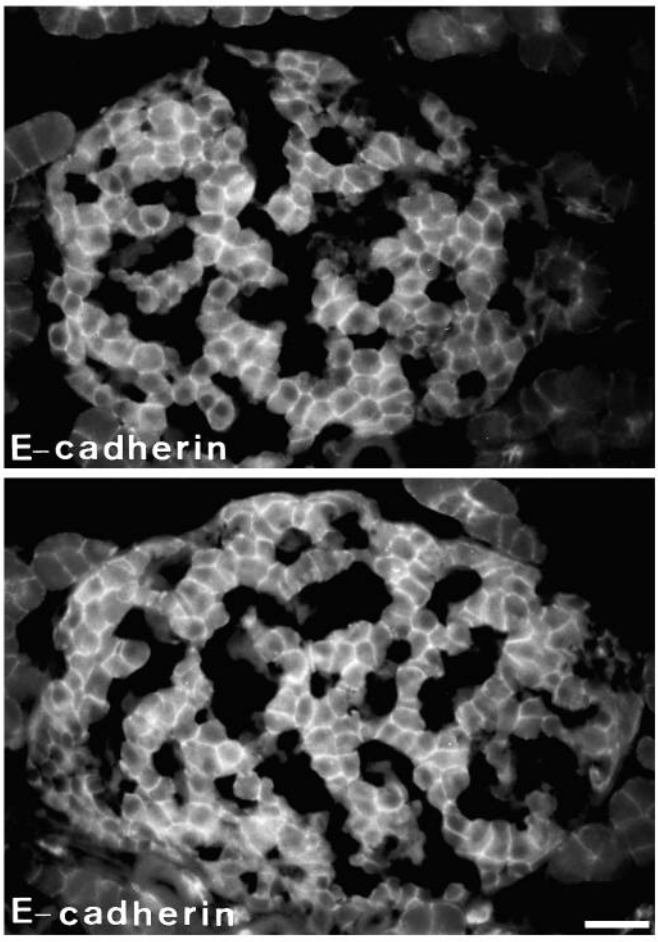

Fig. 1. E-cadherin identification on pancreatic islets. E-cadherin expression was revealed on sections (8 μm) of rat islets from the splenic (upper panel) and the duodenal part (lower panel) of the pancreas by indirect immunofluorescence using a specific anti E-cadherin antiserum as first antibody. E-cadherin appears equally expressed at the surface of all islet cells, in both pancreatic regions. E-cadherin is also detected on acinar cells of the exocrine pancreas. Bar, 30 μm.

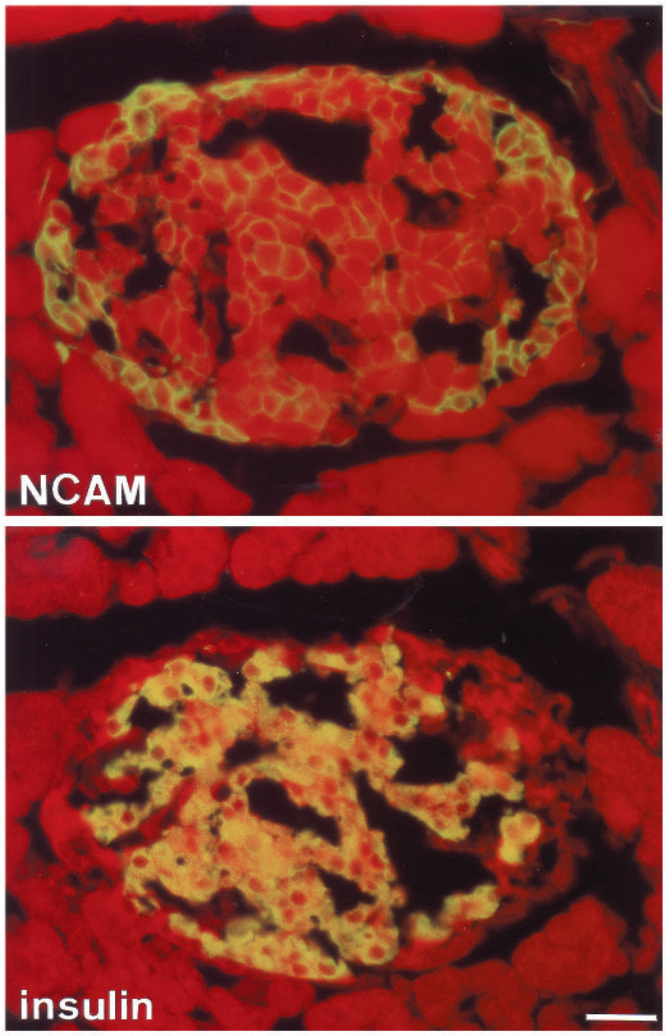

Fig. 2. N-CAM identification on pancreatic islets. Consecutive sections of the pancreas were stained for N-CAM (upper panel) and insulin (lower panel) by indirect immunofluorescence. The intensity of N-CAM labeling is much stronger on cells located at the islet periphery, corresponding to cells devoid of insulin immunoreactivity (non-B-cells). Exocrine cells do not stain for N-CAM. Bar, 30 μm.

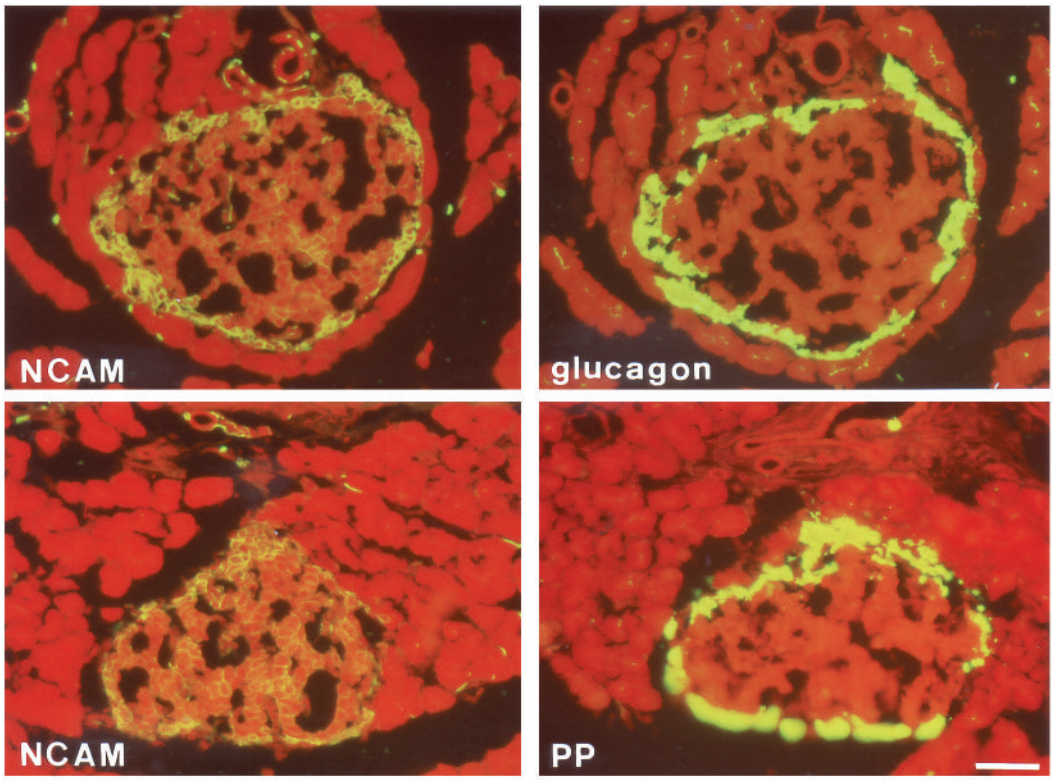

Fig. 3. N-CAM identification on pancreatic islets. Comparison between splenic and duodenal pancreatic regions. Upper panels: consecutive sections from an islet of the splenic part of the pancreas were stained for N-CAM (left) and glucagon (right), demonstrating that the peripheral cells showing stronger immunoreactivity for N-CAM correspond to glucagon-containing cells. Lower panels: consecutive sections from the duodenal part of the pancreas stained for N-CAM (left) and pancreatic polypeptide (right). In this region the peripheral cells showing stronger immunoreactivity for N-CAM can be identified as PP-containing cells. Bar, 60 μm.

previously shown, the peripheral regions of islets from the splenic part of the pancreas contain numerous glucagon-secreting cells and few PP-producing cells, while islets from the duodenal part of the pancreas show a reverse pattern (Orci et al., 1976a). In order to identify the nature of the peripheral cells strongly immunofluorescent for N-CAM, serial sections of splenic and duodenal islets were incubated with anti-N-CAM antibody and either anti-glucagon or anti-PP antibodies. The majority of cells strongly positive for N-CAM in a splenic islet corresponded to glucagon-containing cells (Fig. 3, upper right), while they could be identified as PP cells in a duodenal islet (Fig. 3, lower right). Due to their paucity and their intermediate position between other non-B-cells, it was not possible to clearly evaluate the expression of N-CAM on somatostatin-containing cells. These results demonstrate a stronger N-CAM immunofluorescence on cells located at the islet periphery, irrespective of their secretory type or embryonic origin, suggesting that the difference in N-CAM expression may be responsible for islet cell type segregation.

Expression of E-cadherin and N-CAM on pseudoislets

Sorted islet B- and non-B-cells were mixed in culture, and allowed to form pseudoislets. After 5 days in culture (Fig. 4), indirect immunofluorescence studies on serial sections confirmed that non-B-cells are found at the periphery of the aggregates, and demonstrated that the distribution of CAMs was not modified by the in vitro conditions, inasmuch as N-CAM immunofluorescence predominated on peripheral cells (upper left), corresponding to non-B-cells (lower left) or glucagon-secreting cells (lower right), whereas E-cadherin was similarly expressed on the entire pseudoislet section (upper right). These results further suggest that pseudoislets might represent a good in vitro model for testing a possible role of N-CAM in islet cell type organization.

Role of N-CAM in sorting-out of islet cell types

Sorted islet B- and non-B-cells were labeled with the carbocyanines DiI and DiO, respectively, and mixed in culture in the presence of control or anti-N-CAM Fab fragments. Conventional light microscopy showed that compact pseudoislets have formed both in the control (Fig. 5, upper left), and in the presence of anti-N-CAM Fab fragments (Fig. 5, upper right). Intra-islet organization of the fluoro-labeled cells was studied by confocal microscopy, focused on the equatorial region of each pseudoislet. The figure is representative of a total of four separate experiments. In each experiment, between 20 and 30 pseudoislets were studied in both control and perturbed conditions, giving invariably the same results. Only those aggregates displaying evidence of late fusion were excluded from the study because of active reorganization, as previously observed (Halban et al., 1987). In the presence of non-immune Fab fragments, the characteristic segregation between B-cells (in red) and non-B-cells (in green) occured normally (Fig. 5, lower left). The same result was observed when non-CAM related anti-islet Fab fragments were used (data not shown). In contrast, in the presence of anti-N-CAM Fab fragments, cell type organization was highly perturbed, with small groups of either B- or non-B-cells randomly distributed within pseudoislets (Fig. 5, lower right). Quantitative analysis at the equatorial plan showed that in the control 86% $\pm$ 1.6 of the total green surface was located in the outer third region, compared to only 39.5% $\pm$ 4.7 in the aggregates formed in the presence of anti-N-CAM Fab fragments ($P<0.005$, $n=4$). This

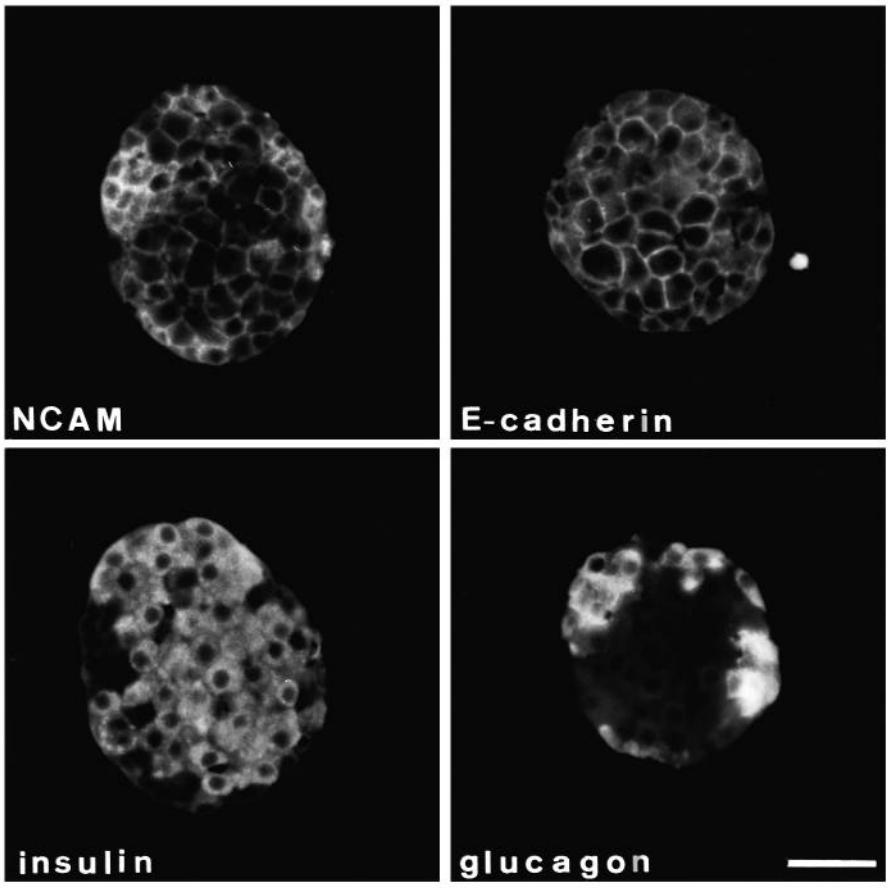

Fig. 4. N-CAM and E-cadherin identification on pseudoislets. Sorted islet B-cells and non-B-cells were mixed together and allowed to form aggregates in culture over a 5-day period. At day 5, several pseudoislets were obtained, and all of them fixed and prepared for cryomicrotomy. Consecutive sections, from randomly selected pseudoislets, were stained for N-CAM, E-cadherin, insulin and glucagon, demonstrating that cells showing stronger immunoreactivity for N-CAM were peripheral non-B-cells, corresponding to glucagon-containing cells. E-cadherin expression appeared identical on the entire islet section. The similarity to the in situ situation suggests that pseudoislets might be a good model in which to study the role of CAMs in the formation and maintenance of islet architecture. The figure shows a representative experiment of several pseudoislets studied from two independent experiments. Bar, 60 μm.

observation provides strong evidence for a critical role of N-CAM in islet cell type segregation.

DISCUSSION

The present studies show that N-CAM is differentially expressed on islet cell types in situ, and provide the first functional evidence that N-CAM-mediated cell-cell interactions play a critical role in islet cell type segregation and organization in vitro.

Previous immunocytochemical studies on sections of adult rat pancreas have shown that N-CAM is restricted to endocrine cells (Langley et al., 1989). In the present study, we confirm that N-CAM immunostaining is present on endocrine cells of the pancreas, and that with the exception of nerve fibers and ganglia, no labeling for N-CAM was detected in acini or ducts. By contrast, E-cadherin immunofluorescence was present on both exocrine and endocrine tissue. The restriction of N-CAM to endocrine cells in adult rat pancreas is in accordance with results in the chick, where N-CAM disappears from exocrine pancreas after differentiation, leaving only LCAM (a close relative to E-cadherin) on acinar cells (Edelman et al., 1983). Little information is available on CAM expression during rat islet ontogenesis. Moller et al. (1992) reported that immunostaining of stage E19 fetal rat pancreas with an antibody specific for the exocrine tissue was excluded from regions stained with N-CAM antibody, demonstrating that at that stage, N-CAM expression is already restricted to the developing endocrine pancreas.

There is some controversy regarding a possible topographical modulation of N-CAM expression within rat islets. Immunoflow cytometry and immunoblotting on sorted populations of islet cells revealed higher levels of N-CAM on islet non-B-cells, as compared to B-cells (Rouiller et al., 1990). In situ, either stronger staining in peripheral islet cells (Moller et al., 1992) or no apparent difference in staining between cells of the core and cells of the islet periphery (Langley et al., 1989) has been reported. Differences in embryonic origin of the tissue studied might be responsible for the discrepancy. In the present study, serial sections of the islets demonstrate that the intense peripheral N-CAM labeling corresponds to glucagon cells in the splenic part of the pancreas, and to PP-cells in islets of the duodenal part. N-CAM and E-cadherin staining were at times performed on serial sections, thus excluding the possibility that the differential N-CAM immunostaining might be due to differences in antibody access to the cells. The modulation of N-CAM expression according to the distribution of the cell type within the islet, irrespective of its secretory type (glucagon or PP) or embryonic origin (dorsal or ventral bud), suggests that it may play a fundamental role in islet cell type segregation.

Islet B- and non-B-cells, sorted by flow cytometry, and differentially labeled with carbocyanines, when mixed in culture and allowed to stay in suspension, form compact aggregates with smooth borders; cell types are first randomly distributed

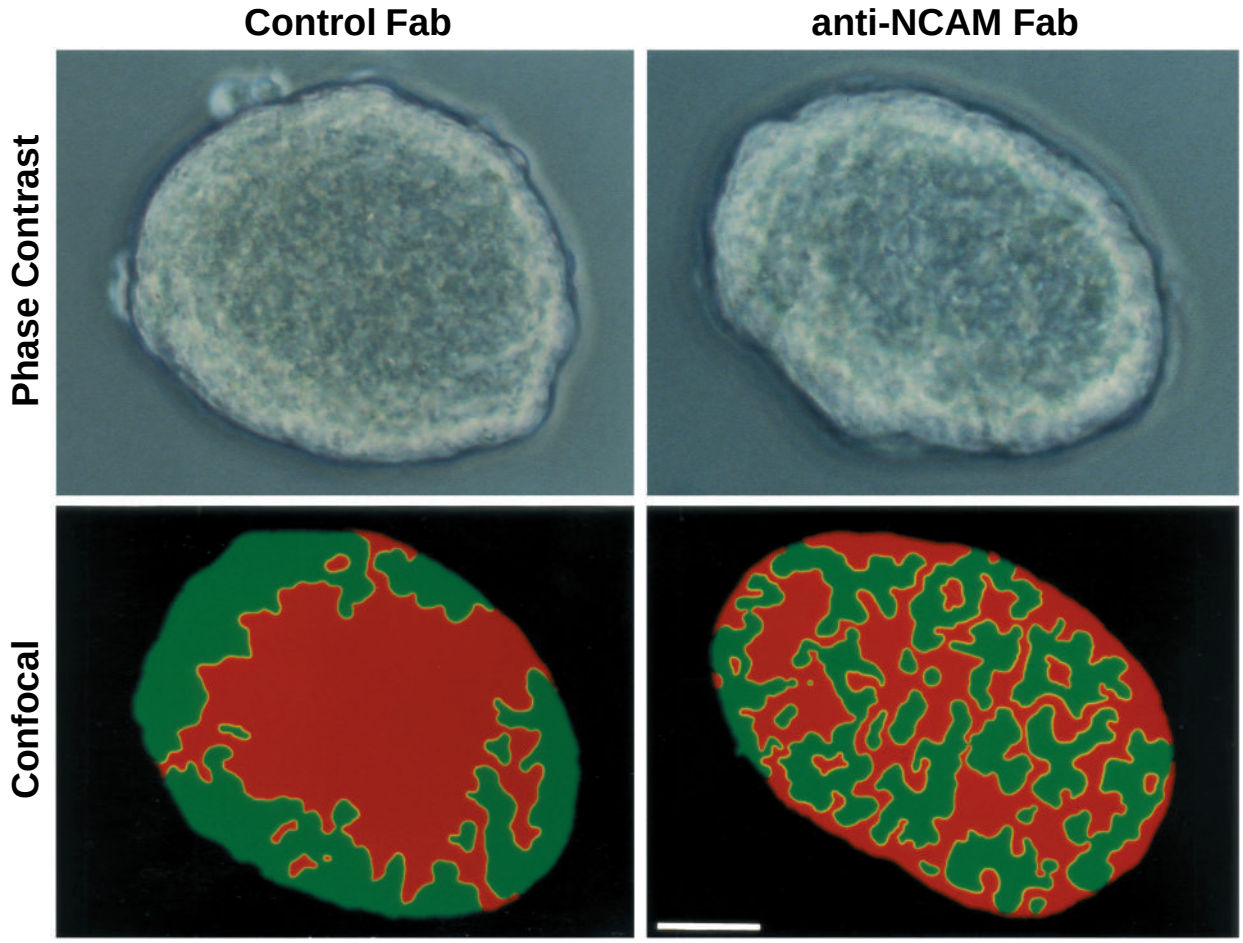

Fig. 5. Pseudoislet segregation assay. Sorted islet B-cells and non-B-cells were labeled with the fluorescent carbocyanines DiI and DiO, respectively. The two differentially labeled populations were then mixed together and allowed to form aggregates in culture over a 5-day period. At day 5, control cells had formed compact pseudoislets with smooth borders, as demonstrated by conventional phase-contrast microscopy (upper left). Under confocal fluorescence microscopy, the characteristic sorting-out of cells can be appreciated (lower left), with B-cells in the center (red fluorescence), surrounded by non-B-cells (green fluorescence). In cells cultured for 5 days in the presence of an anti-N-CAM Fab fragment, aggregates displayed a similar appearance to that in the control when viewed by phase-contrast microscopy (upper right), but the typical organization between B- and non-B-cells was highly perturbed (lower right), supporting a role for N-CAM in cell-type organization. The figure is representative of the situation observed in 4 separate experiments. Bar, 40 μm.

within aggregates, then segregation occurs, with a peripheral distribution of non-B-cells, reminiscent of native islets (pseudoislets). This behavior in culture has been shown both with embryonic (Montesano et al., 1983) and postmitotic islet cells (Tze and Tai, 1982; Hopcroft et al., 1985; Halban et al., 1987), and is in accordance with the classical observations of Wilson (1907), Holtfreter (1939) or Armstrong (1989) that dispersed cells of a particular tissue have the ability to reunite and reconstitute a functional tissue. In the 5-day pseudoislets, N-CAM and E-cadherin displayed the same pattern of expression that islets in situ, i.e. with a higher expression of N-CAM on non-B-cells, compared to B-cells, and a similar expression of E-cadherin on both cell types, thus legitimizing the use of pseudoislets as a reliable in vitro model to study the possible role of CAMs in islet architecture. Nose et al. (1988) have shown that when two clones of L-cells transfected with different cadherins are mixed together, cells from either clone aggregate independently. When these transfected cells were constrained to intermix in aggregates by adding a lectin, discrete compartments formed over time inside aggregates according to the transfected cadherin. In the case of islets, we previously reported that E-cadherin mediates most if not all Ca^{2+}-dependent adhesion of both B- and non-B-cells. We now show that blocking N-CAM function with a specific polyclonal antibody greatly perturbs cell-type organization, without preventing the formation of compact aggregates. By analogy with Nose's experiments (Nose et al., 1988), our results suggest that E-cadherin provides the cohesive force to cells within aggregates, irrespective of their type, and that differences in expression of N-CAM and possibly other CAMs between cell types are responsible for the segregation. A role for N- and R-cadherins is suggested by the recent detection of differential levels of mRNA between B- and non-B cells, using quantitative PCR analysis (Hutton et al., 1993). This view is supported by the studies of Friedlander et al. (1989) showing that both specificity and amount of cell adhesion molecules are involved in cell sorting-out.

One intriguing observation is that it is the cell population

carrying the higher amounts of CAMs (same level of E-cadherin + higher levels of N-CAM) that segregates to the pseudoislet periphery. According to the model of Steinberg (1963), one condition that needs to be satisfied for a cell population to segregate to the periphery of mixed aggregates is that the homotypic cohesion strength of the peripheral cells should be lower than that of cells of the central population. This model, if correct, would therefore imply that the higher expression of N-CAM on islet non-B-cells, may weaken overall cell-cell adhesion. It is possible that N-CAM interferes in the binding of other CAMs by a steric effect within the intercellular space, as has been demonstrated for the embryonal form of N-CAM, which is rich in polysialic acid (Rutishauser et al., 1988). Alternatively, N-CAM might provide a signal causing cells to migrate and stay at the islet periphery. Studies comparing the cohesion strengths of B- and non-B-cell homotypic adhesion would be the first step towards a better understanding of this interesting issue.

During development of the pancreas, at least two mechanisms can be proposed to explain non-B-cell location to the islet periphery. It is known that when segregation between B and non-B-cells occurs (between E-10.5 and E-19) (Herrera et al., 1991) N-CAM is expressed on pancreatic endocrine cells (Moller et al., 1992). An up-regulation of N-CAM on non-B-cells and/or a down-regulation on B-cells could therefore result in the observed segregation. The latter mechanism is supported by our recent observation that the up-regulation of Ca^{2+}-independent CAMs on B-cells by TNF-α is associated with the inability of B- and non-B-cells to segregate within pseudoislets in vitro (Cirulli et al., 1993). Furthermore, transformed B-cells (RIN-cells), which re-express very high levels of N-CAM (presumably consequent to transformation) (Rouiller et al., 1990), migrate to the periphery of aggregates composed of a mixture of transformed and primary cells (Halban et al., 1988).

Taken together, the data presented here provide evidence for a role of N-CAM in the segregation of islet cell types within pseudoislets in vitro. They support previous work suggesting that islet CAMs may be involved in the establishment and maintenance of islet architecture (Rouiller et al., 1990, 1991). They do not exclude, however, the possibility that other, as yet undefined, morphoregulatory molecules may serve to complement and/or modulate this action of N-CAM.

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