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CONCANAVALIN A BINDING AND CYTODIFFERENTIATION IN PANCREATIC ACINAR CARCINOMA OF RAT

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The binding of concanavalin A to the plasmalemma of acinar carcinoma cells was characterized by electron microscopy utilizing horseradish peroxidase. Heavy labeling due to specific concanavalin A binding was detected on the plasmalemma of undifferentiated carcinoma cells lacking zymogen maturation, neoplastic cells of intermediate differentiation with only occasional zymogen granules, and highly differentiated acinar carcinoma cells containing numerous cytoplasmic zymogen granules. The plasmalemma of acinar carcinoma cells was also compared to the normal pancreatic acinar cell plasmalemma by measurement of specific ^{125}I-labeled concanavalin A binding. Although only about one-third of pancreatic acinar carcinoma cells demonstrate mature zymogen differentiation, the acinar carcinoma had a full complement of normal plasmalemma receptors for ^{125}I-labeled concanavalin A. It is concluded that, unlike normal pancreas, the presence of concanavalin A receptors on the plasmalemma of acinar carcinoma cells is not a specific membrane marker for differentiated cells containing zymogen granules.

Introduction

The pancreatic acinar cell carcinoma of rat demonstrates a spectrum of cellular differentiation ranging from morphological forms totally devoid of zymogen granules to forms containing a full complement of zymogen granules [1]. Lectin reactivity of plasma membranes can provide useful information on the temporal sequence of plasmalemma maturation during normal and neoplastic cell differentiation [2–4]. The appearance of concanavalin A receptors on the plasmalemma of rat pancreatic acinar cells occurs late in normal embryogenesis [5] and can be regarded as a membrane marker for highly differentiated acinar cells containing zymogen granules. Concanavalin A binding properties of acinar carcinoma cells were therefore determined to test whether specific concanavalin A binding correlates with organellar cytodifferentiation in a fashion similar to that reported for normal differentiating acinar cells. Two groups of concanavalin A binding experiments were performed. First, specific concanavalin A binding to acinar carcinoma cells of varying degrees of morphological differentiation was examined by electron microscopy utilizing the peroxidase technique. Second, the binding of ^{125}I-labeled concanavalin A to acinar carcinoma cells was quantitatively compared to binding by fully differentiated acinar cells obtained from normal adult rat pancreas. It has been found that the plasmalemma of undifferentiated carcinoma cells lacking zymogen granules is as heavily labeled by the concanavalin A-peroxidase technique as the plasmalemma of well-differentiated carcinoma cells

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containing zymogen granules, and that the acinar carcinoma possesses a complement of concanavalin A receptors equivalent in number per cell and affinity to normal pancreatic acinar cells. The observed concanavalin A binding properties of acinar carcinoma cells indicate that plasmalemma glycoconjugate patterns specific for concanavalin A binding are dissociated from patterns of zymogen cytodifferentiation in the acinar carcinoma. A preliminary report of this work has appeared [6].

Materials and Methods

Chemicals. Collagenase preparations (CLSPA, lot 50 M 588Y; CLS, lot 49 H 297; type III, lot 41 E 065) and soybean trypsin inhibitor were purchased from Worthington Biochemicals, Freehold, NJ. The type III collagenase was purified by Sephadex G-75 column chromatography [7]. Horseradish peroxidase and albumin (fraction V) were from Sigma Chemical Co., St. Louis, MO. The albumin was extensively dialyzed against distilled water and lyophilized prior to use. Methyl-α-D-mannopyranoside was from Pfanstiehl Laboratories, Waukegan, IL. Concanavalin A purified by chromatography on Sephadex was obtained from Pharmacia, Piscataway, NJ. Carrier-free $Na^{125}I$ was obtained from New England Nuclear (Boston, MA) in aqueous solution. Concanavalin A was labeled with ^{125}I by the chloramine T method as described by Gordon and Young [8]. A specific activity of 57500 and 74023 cpm/μg protein was determined for the two ^{125}I-labeled concanavalin A preparations utilized in the present study.

Dissociation of cells. Dissociation of acinar carcinoma and normal pancreas was performed in a modified Krebs-Ringer buffer containing 125 mM NaCl, 6.2 mM KCl, 33 mM $NaHCO_3$, 1.5 mM KH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 18.2 mM glucose, 1.0% (v/v) complete amino acid supplement (Kansas City Biologicals, Lenexa, KS), 2.0 mM glutamine, albumin fraction V (2 mg/ml), soybean trypsin inhibitor (0.1 mg/ml), 100 U/ml penicillin, and 100 μg/ml streptomycin. The buffer was maintained at pH 7.4 when supplemented with 2.0 mM Hepes. Fragments obtained by mechanical disruption of the acinar carcinoma as previously described [9] were incubated at 37°C with collagenase (200 units/ml) for 90 min. At completion of the incubation with collagenase, the cell preparation was washed by centrifugation in 4% albumin, passed through a 60 μm nylon mesh, and drawn 5 times through a serological pipette (TD Kimax, catalog no. 37033, internal diameter of 1.2 mm) and a Pasteur pipette tapered by flaming. Dissociated normal acinar cells were obtained by the technique of Amsterdam et al. [10]. Dissociation into single acinar cells with depletion of erythrocytes was confirmed by microscopic examination. If necessary cell preparations were further washed with 4% albumin to remove all erythrocytes. For ^{125}I-labeled concanavalin A binding experiments approximately one-half of the acinar carcinoma or normal acinar cells were thrice washed and suspended in the modified Krebs-Ringer buffer containing no glucose. The remaining one-half of acinar cells was washed and suspended in the same Krebs-Ringer buffer containing 0.2 M methyl-α-D-mannopyranoside.

Concanavalin A-peroxidase method. Dissociated acinar carcinoma cells ($5 \cdot 10^6$ cells/ml) were fixed for 1 h with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. After fixation, the cells were rinsed in buffer solution and incubated with 100 μg/ml of concanavalin A in cacodylate buffer for 30 min at room temperature. The cells were rinsed in cacodylate buffer, suspended in buffer containing 200 μg/ml of horseradish peroxidase for 1 h at room temperature, and were then processed for electron microscopy as previously described [11]. Control samples were processed in identical fashion except that methyl-α-D-mannopyranoside was added to the concanavalin A solution.

Measurement of ^{125}I-labeled concanavalin A binding to acinar cells. Non-specific binding of ^{125}I-labeled concanavalin A to acinar cells was measured in buffer containing 0.2 M methyl-α-D-mannopyranoside and total binding in buffer without added mannopyranoside. Specific binding of ^{125}I-labeled concanavalin A was then calculated as the difference between total and non-specific binding. Specific ^{125}I-labeled concanavalin A binding generally constituted 80–95% of total ^{125}I-labeled concanavalin A binding detected. All binding experiments were performed at 4°C in Krebs-Ringer buffer containing Ca^{2+}, Mg^{2+}, amino acids, antibiotics, and albumin. To measure binding, suspensions of acinar carcinoma or normal

acinar cells were added to polystyrene tubes (Kimble, Toledo, OH), cooled to 4°C for 30 min, and ^{125}I-labeled concanavalin A added to give a final reaction mixture volume of 1.0 ml. Following incubation with ^{125}I-labeled concanavalin A each reaction mixture was layered over a 1.0 ml cushion of 4% albumin (which contained 0.2 M mannopyranoside in tubes measuring non-specific binding), centrifuged at 150 × *g* for 3 min, and the resulting supernatant carefully separated from the cell pellet by aspiration with a Pasteur pipet. The cell pellet was suspended in the appropriate Krebs-Ringer buffer (without or with mannopyranoside), layered over a second albumin cushion, centrifuged at 150 × *g* for 3 min, and the supernatant separated from the cell pellet. The cell-bound radioactivity was measured in a γ-counter (Searle model 1285). In addition, the radioactivities of the supernatants obtained from the first and second albumin washes were measured in the γ-counter. Values for free ^{125}I-labeled concanavalin A utilized in Scatchard plot analysis were derived from measured radioactivities of the first and second albumin washes. The specific radioactivity of ^{125}I-labeled concanavalin A was appropriately corrected for isotope decay in all calculations.

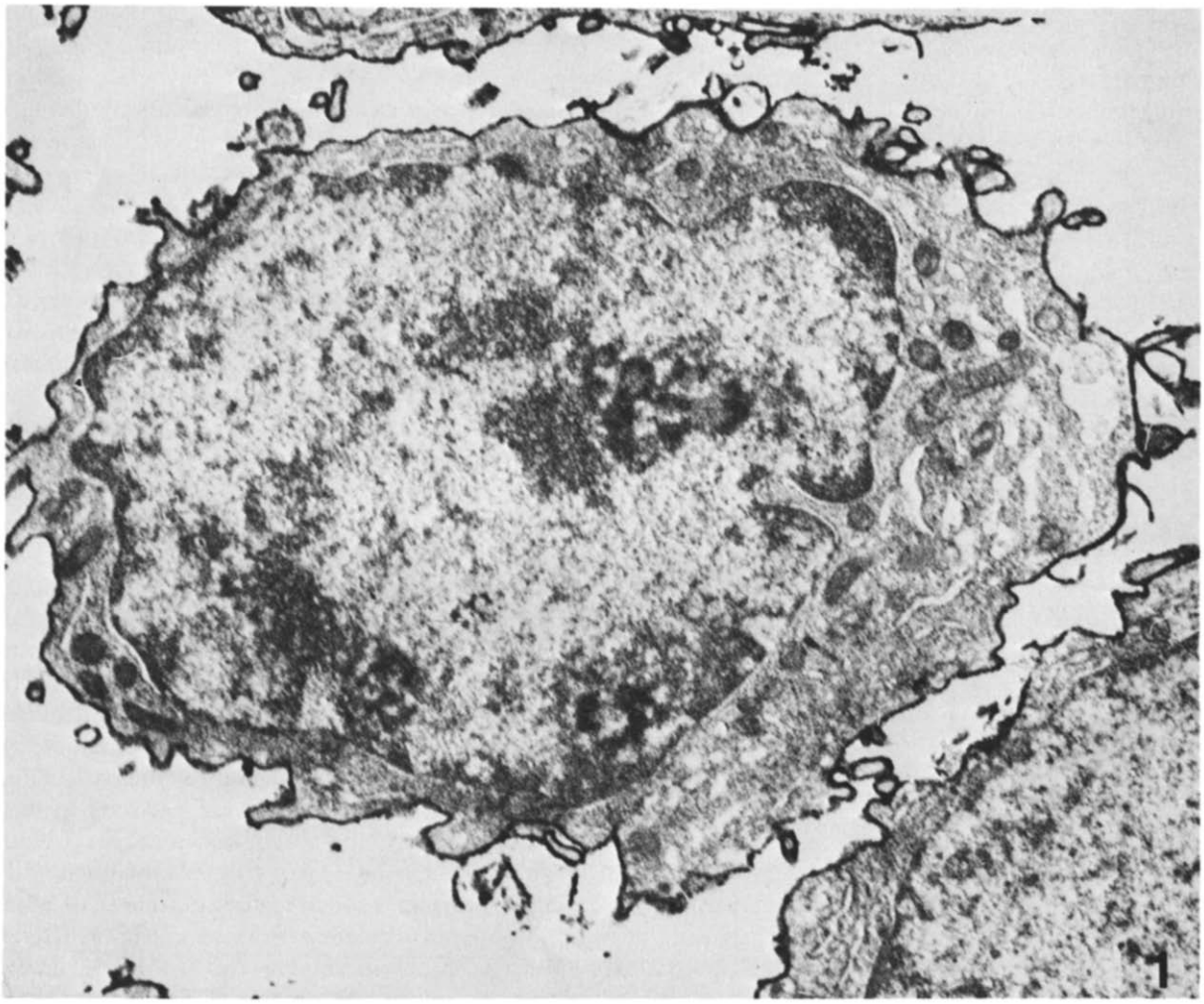

Fig. 1. Undifferentiated acinar carcinoma cell labeled by the concanavalin A-peroxidase technique. ×14000.

Results

In the initial group of experiments, specific binding of concanavalin A to the plasmalemma of acinar carcinoma cells was visualized by electron microscopy utilizing the peroxidase technique [11]. Continuous labeling of membrane surfaces with electron dense reaction product was observed for undifferentiated carcinoma cells lacking zymogen maturation (Fig. 1), for acinar carcinoma cells of intermediate differentiation with nuclear polarity and abundant rough endoplasmic reticulum but only occasional zymogen granules (Fig. 2), and for highly differentiated acinar carcinoma cells containing numerous mature zymogen granules (Fig. 3). Control preparations with methyl-α-D-mannopyranoside were negative. Concanavalin A receptors are present in the plasmalemma of acinar carcinoma cells, therefore, regardless of the degree of cytodifferentiation of the carcinoma cells.

Concanavalin A binding by acinar carcinoma cells was quantitatively compared in the next group of experiments to concanavalin A binding by normal pancreatic acinar cells. Measurement of ^{125}I-labeled concanavalin A binding was utilized in these comparative experiments. The effect of incubation time with ^{125}I-labeled concanavalin A and the number of cells on specific concanavalin

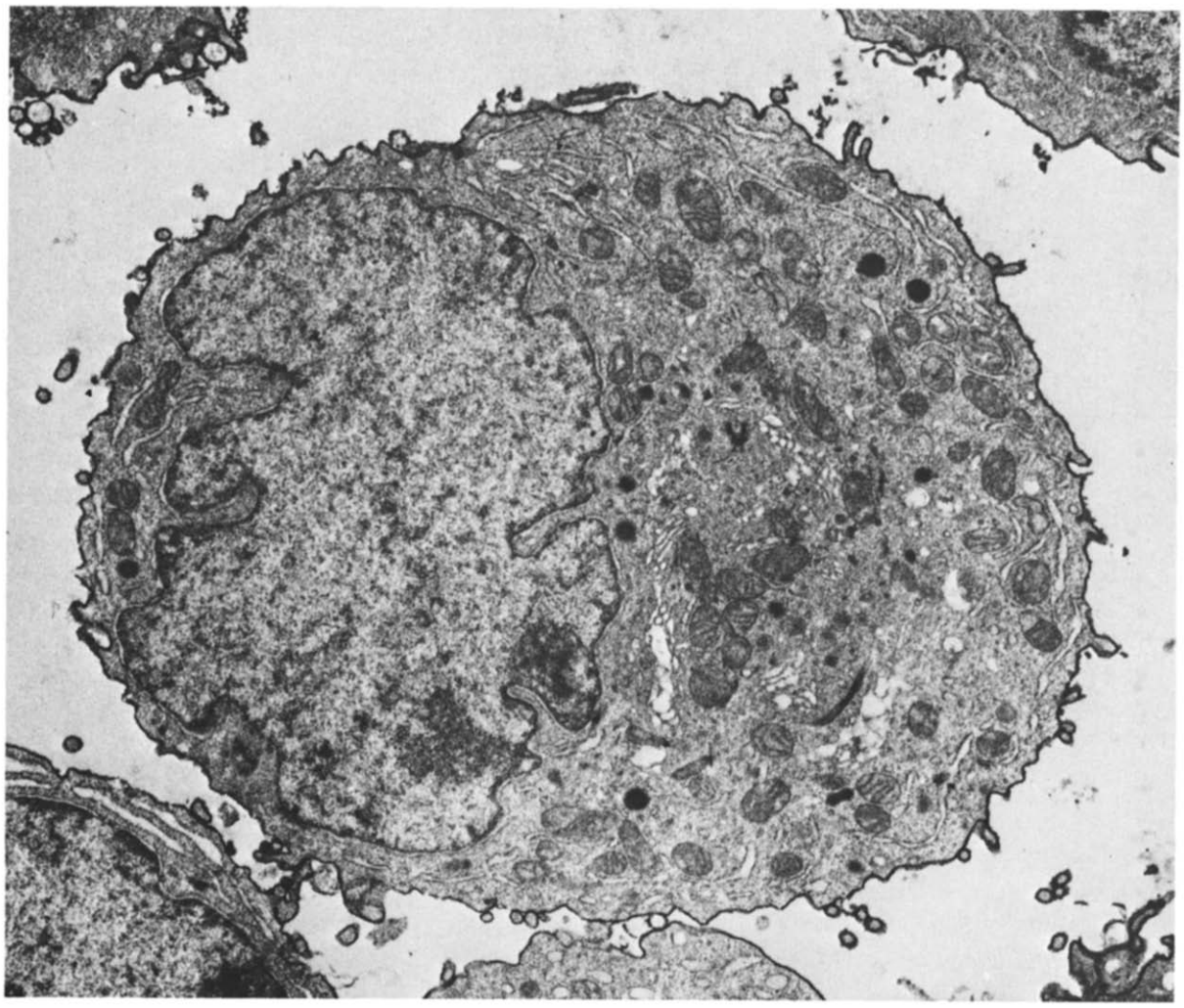

Fig. 2. Acinar carcinoma cell of intermediate differentiation labeled by the concanavalin A-peroxidase technique. ×10800.

A binding to acinar carcinoma cells was initially established. Specific binding of ^{125}I-labeled concanavalin A to acinar carcinoma cells reached equilibrium within 30 min and the extent of specific concanavalin A binding per carcinoma cell was independent of cell number up to $6 \cdot 10^6$ cells/ml (data not shown). To measure specific ^{125}I-labeled concanavalin A binding to acinar carcinoma cells under equilibrium conditions, therefore, $6 \cdot 10^6$ carcinoma cells/ml or less were incubated with ^{125}I-labeled concanavalin A for 2 h. The binding curve obtained under equilibrium conditions in four separate experiments with acinar carcinoma cells is shown in Fig. 4. It is seen that ^{125}I-labeled concanavalin A specifically binds to acinar carcinoma cells in a highly concentration-dependent fashion up to 300 μg/ml of added concanavalin A. In addition, the Scatchard plot [12] derived from specific ^{125}I-labeled concanavalin A binding data over the entire concentration range of added ^{125}I-labeled concanavalin A is linear (inset, Fig. 4). The linear Scatchard plot in the inset of Fig. 4 represents the results of a single binding experiment and gives values of $4.4 \cdot 10^7$ for the maximum number n and of $2.4 \cdot 10^5$ for the association constant K_a of the plasmalemma concanavalin A

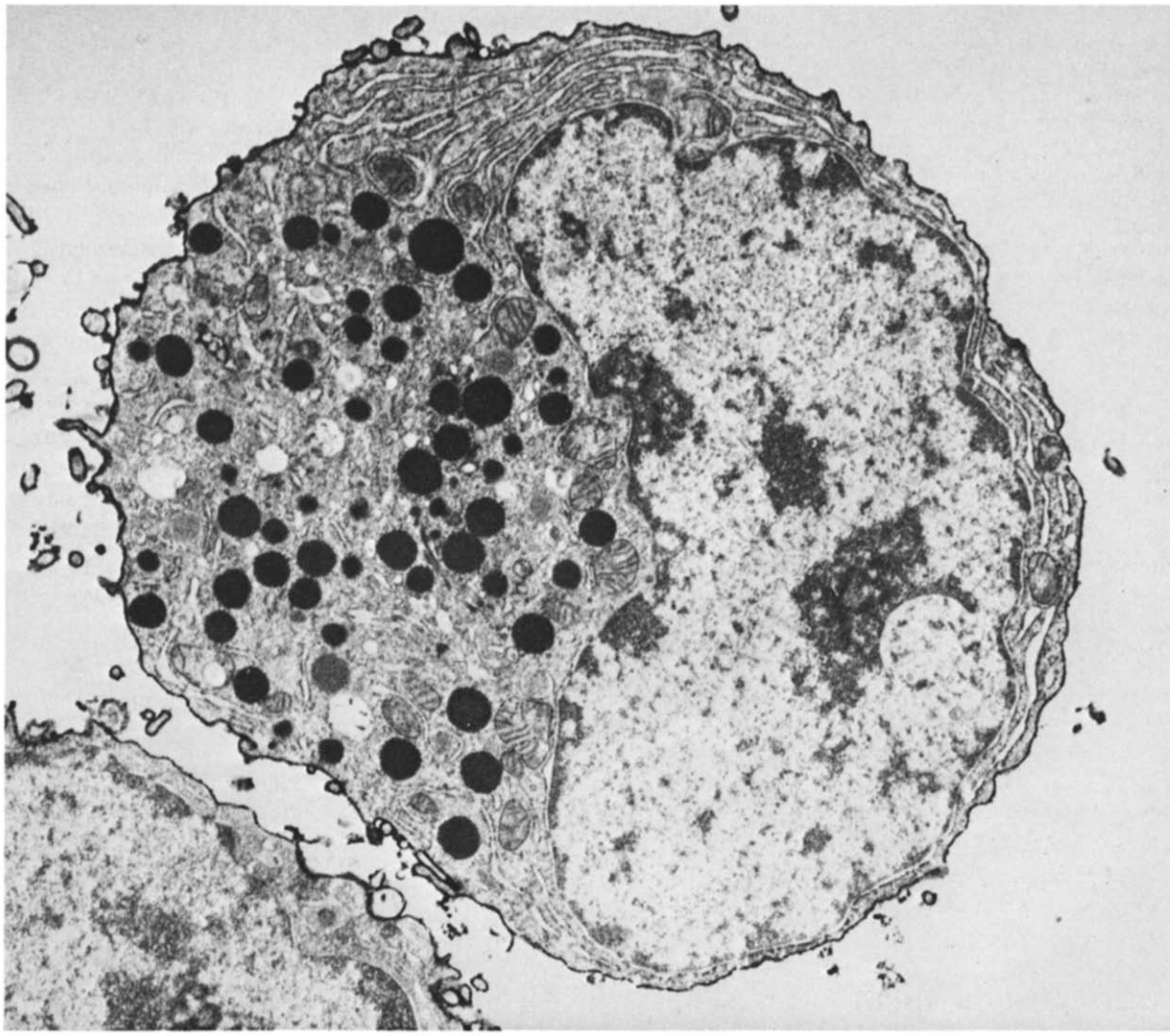

Fig. 3. Well-differentiated acinar carcinoma cell labeled by the concanavalin A-peroxidase technique. ×9600.

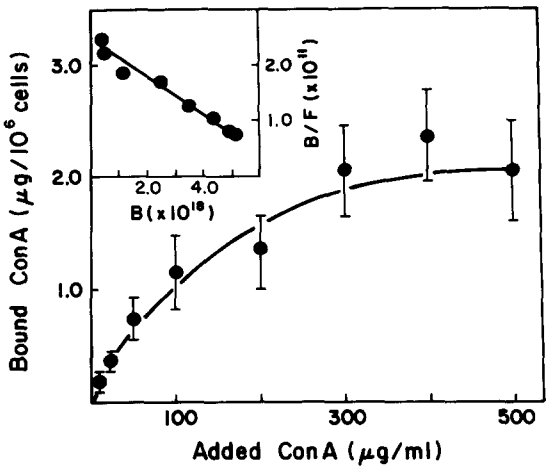

Fig. 4. Specific binding of ^{125}I-labeled concanavalin A to acinar carcinoma cells as a function of added ^{125}I-labeled concanavalin A concentration. Each point represents the mean ±S.E. observed in four experiments at the indicated concentrations of added ^{125}I-labeled concanavalin A. Inset. Scatchard plot analysis of specific ^{125}I-labeled concanavalin A binding to acinar carcinoma cells. The number of molecules of concanavalin A bound to each acinar carcinoma cell is represented on the abscissa and the ratio of the bound to free (unbound) concanavalin A on the ordinate. A molecular weight of 55000 for dimeric concanavalin A was utilized in the Scatchard plot analysis.

receptors. Scatchard plot analysis for each of the four experiments reported in Fig. 4 yielded an average value of $3.4 \cdot 10^7$ for n and $4.1 \cdot 10^5$ for K_a. The ^{125}I-labeled concanavalin A binding data of Fig. 4 were obtained using carcinoma cells dissociated with collagenase purified by Sephadex G-75 chromatography [7]. We confirmed by measurement of non-collagenolytic protease activity using casein as substrate [13] that purification of collagenase by Sephadex G-75 chromatography completely removed the non-collagenolytic proteases. We should note that in a separate group of experiments the acinar carcinoma was dissociated into single cells by commercial collagenase preparations (CLSPA and CLS) which were not purified in our laboratory. Scatchard analysis of three ^{125}I-labeled concanavalin A binding experiments performed with these carcinoma cells resulted in values of $2.9 \cdot 10^7$ for n and $1.8 \cdot 10^5$ for K_a. It appears, therefore, that non-collagenolytic proteases present in commercial preparations of collagenase have no detectable effect on concanavalin A binding properties of acinar carcinoma cells.

Specific binding of ^{125}I-labeled concanavalin A to normal pancreatic acinar cells was measured under reaction conditions identical to those utilized for acinar carcinoma cells. Linear Scatchard plots similar to the plot shown for acinar carcinoma cells (inset, Fig. 4) were obtained for normal pancreatic acinar cells in three separate experiments (data not shown). Values of $3.1 \cdot 10^7$ for n and of $0.8 \cdot 10^5$ for K_a of concanavalin A receptor sites on normal acinar cells were calculated by Scatchard analysis. Values reported here for the total number and affinity of concanavalin A receptor sites on normal pancreatic acinar cells are in close agreement with values reported by Maylié-Pfenninger and Jamieson [14]. In addition, no significant difference exists between the number and affinity of plasmalemma concanavalin A receptor sites of acinar carcinoma and normal acinar cells.

A small difference in the plasmalemma density of concanavalin A receptors was noted between acinar carcinoma and normal acinar cells. Utilizing the cell surface area of acinar carcinoma (249 μm^2) and normal acinar cells (583 μm^2) [11] and the total number of concanavalin A receptors determined in the present study per acinar carcinoma and normal acinar cell, calculated values for concanavalin A receptor density on acinar carcinoma and normal cells are $1.37 \cdot 10^5$ and $0.58 \cdot 10^5$ μm^{-2}, respectively. The 2-fold increase in plasmalemma density of concanavalin A receptors apparent for transformed pancreatic acinar cells is much less than the several-fold increase (3.5–7-times) which has been reported by some investigators for other types of transformed cells [15,16].

Discussion

The temporal sequence of plasmalemmal differentiation has been established in normal embryogenesis of the rat pancreas using different lectins, including concanavalin A, as probes for cell surface glycoconjugates [5]. At day 15 in utero the embryonic pancreas consists of cords of morphologically undifferentiated epithelial cells (the protodifferentiated stage) whose plasmalemmas do not contain receptors for concanavalin A. The day 17 embryonic pancreas (secondary transition stage) demonstrates acini composed of epithelial cells

containing markedly increased endoplasmic reticulum and a few small zymogen granules. However, epithelial cells comprising acini are devoid of plasmalemma receptors for concanavalin A. Concanavalin A binding was not observed until day 19 in utero (the prenatal stage) when acinar cytodifferentiation has been accomplished, with acinar cells containing large numbers of cytoplasmic zymogen granules. Morphological analysis by electron microscopy of dissociated acinar carcinoma cells has revealed undifferentiated cells displaying few rough endoplasmic reticulum profiles and no zymogen granules, cells of intermediate (partial) differentiation with abundant stacks of rough endoplasmic reticulum but no or only occasional immature zymogen granules, and highly differentiated carcinoma cells with abundant well-formed zymogen granules [1]. However, in contrast to normal pancreatic embryogenesis, concanavalin A receptors were detected by electron microscopy in the present study on the plasmalemma of undifferentiated and partially differentiated carcinoma cells as well as highly differentiated carcinoma cells. Also, despite the fact that highly differentiated granule-containing cells constitute only one-third the total number of acinar carcinoma cells [1], no quantitative evidence was obtained from ^{125}I-labeled concanavalin A binding data for diminished concanavalin A binding to carcinoma as compared to normal acinar cells. Single cell preparations obtained from adult pancreas consist predominantly (approx. 95%) of zymogen granule-containing acinar cells. It is concluded, therefore, that plasmalemma glycoconjugate patterns specific for concanavalin A binding do not correlate with patterns of organellar cytodifferentiation in the fashion described for normal differentiating pancreatic acinar cells [5]. Mechanisms to explain our finding that concanavalin A binding is not restricted to acinar carcinoma cells with zymogen maturation remain to be determined. It can be proposed that genetic programs for regulation of plasma membrane maturation are dissociated from programs for organellar cytodifferentiation in the acinar carcinoma. Recent results obtained by bromodeoxyuridine treatment of embryonic pancreatic rudiments indicate that the genetic program for differentiation of plasmalemma glycoconjugates is independent of differentiation programs for intracellular organelles [17]. Such a genetic dissociation in the pancreatic carcinoma could result in the acquisition by undifferentiated cells of concanavalin A receptors. Additional lectins of known differential reactivity toward undifferentiated and differentiated cells of the pancreas [5] are presently being utilized in our laboratory to further probe patterns and mechanisms of membrane differentiation in pancreatic cancer.

Acknowledgments

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