

THE ABSENCE OF H-2 ANTIGENS FROM
MOUSE PANCREATIC β -CELLS
DEMONSTRATED BY IMMUNOFERRITIN LABELING*

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Antigens of the mouse H-2 locus have been studied by immunoferritin labeling on a variety of dissociated epithelial cells (1, 2).¹ Most of the epithelial cells were dissociated by EDTA and trypsin after prefixation of the tissues in periodate-lysine-paraformaldehyde. Prefixation immobilized H-2 antigens in their native positions and preserved the shape of the dissociated epithelial cells. Neither the fixation nor the trypsin treatment had any effect on H-2-antigen labeling.

About one-half of the epithelial cell types studied expressed H-2 antigens on their lateral and basal membranes but not on apical membranes. These included: the lining epithelial cells of the duodenum-jejunum, ileum, bile duct, gall bladder, uterus, and tracheal brush cells. The remainder of the epithelial cells did not express H-2 antigens on any part of their plasma membranes. These included: the ciliated cells of the trachea, hepatocytes, the lining epithelium of the vas deferens, and parietal cells and chief cells of the stomach. The absence of H-2 antigens from a significant number of normal mouse epithelial cells suggested a study of the expression of this locus on an epithelial cell whose transplantation is of clinical interest; namely, the pancreatic β -cell.

Materials and Methods

Mice and Antiserum. The mouse strains used were C57BL/10Sn and B10.BR/SgSn from The Jackson Laboratory, Bar Harbor, Maine, and C3H/HeJ from The Institute of Medical and Veterinary Sciences, Adelaide, Australia. Alloantiserum specific for the H-2^b locus was produced in B10.BR/SgSn \times C3H/HeJ hybrid females by immunizing with C57BL/10Sn spleen cells. Six immunizations were given at weekly intervals, beginning with 0.05 spleen per mouse and ending with 0.3 spleen per mouse. Antiserum titers of \cong 1/60 were demonstrated by hemolysis assay (3) with rabbit complement (4).

Preparation of Islets of Langerhans. Islets of Langerhans were isolated essentially by the method of Lacy and Kostianovsky (5). Pancreases were collected in batches of 10, minced finely, and suspended in Hanks' balanced salt solution at 0°C. Fatty tissue rose to the surface and was removed by pipet. Pancreatic tissue was then digested with 10 mg of collagenase (Sigma Chemical Co., St. Louis, Mo., type V) in 10 ml of Hanks' balanced salt solution at 37°C. The suspension was agitated by hand for \cong 8 min, then centrifuged at 400 g for 2 min. The pellet was resuspended in another 10 ml of the collagenase solution and again agitated at 37°C until

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¹ Parr, E. L., and W. N. Kirby. 1978. An immunoferritin labeling study of H-2 antigens on dissociated epithelial cells. *J. Histochem. Cytochem.* In press.

digestion was complete. After collection of the islets by brief centrifugation, they were partially separated from the lighter tissue debris by allowing them to sediment four times at unit gravity for 4 min for 10 ml of Hanks' balanced salt solution. Islets were further separated from the tissue debris by pipetting or by Ficoll-gradient sedimentation (Sigma Chemical Co., St. Louis, Mo.). In the latter case, the digest was suspended in 23% Ficoll in Hanks' solution, overlaid by 25% Ficoll, and overlaid with 20% Ficoll. After centrifugation for 10 min at 400 *g*, the 23% layer and both interfaces were collected, diluted with Hanks' balanced salt solution, and centrifuged again to sediment the islets. The small amount of nonislet tissue debris present at this stage was removed later. To collect islets by pipetting, the tissue digest was suspended in phosphate-buffered saline (PBS)² containing 1% bovine serum albumin (BSA), and examined with a dissecting microscope. Islets recovered from the digest by pipet were washed once in this medium before further processing. Two C57BL/10Sn islet preparations, each from 10 pancreases, were obtained by pipetting and two by Ficoll-gradient sedimentation; one B10.BR/SgSn islet preparation was obtained by each method for control. The six islet preparations were prefixed in periodate-lysine-paraformaldehyde (6) (PLP) for 3 h at 4°C. The fixative had a higher osmotic strength than the one originally described. It was made by combining 3 ml of 0.10 M lysine in 0.10 M phosphate buffer with 1 ml of 4% paraformaldehyde and 9 mg of periodic acid. One additional C57BL/10Sn islet preparation was obtained by Ficoll sedimentation and washed in PBS without prefixation in PLP.

Dissociation of Islets of Langerhans. All islet preparations were washed for 20 min in PBS containing 0.02% EDTA and dissociated by exposure to 0.25% trypsin (Sigma Chemical Co., type III) in the same medium for 15 min at 37°C. The islets and free cells were collected by centrifugation and further dispersed by pipetting briefly in cold 5% BSA in PBS. The islets dissociated readily to provide a suspension of single cells and small cell clusters. These were collected by centrifugation and washed in 10% nonimmune rabbit serum in PBS for 30 min at 4°C before labeling. At this stage, undissociated tissue debris was allowed to sediment at 1 *g* and was then removed by pipet.

Antibody-Ferritin Conjugate. Rabbit anti-mouse gamma globulin was prepared as described by Perkins et al. (7). The IgG fraction of this antiserum was isolated on a protein A column and conjugated to ferritin with glutaraldehyde in a two-step procedure as described previously (2), except that ferritin was activated at a lower concentration (5 mg/ml) with a larger amount of glutaraldehyde (8 mg/100 mg of ferritin). This modification reduced ferritin aggregation in the conjugate and improved labeling specificity.

Labeling Procedure. Pellets of islet cells obtained from 10 pancreases were <1 μ l in volume. They were suspended in 200 μ l of a 1/10 dilution of mouse antiserum in 10% rabbit serum for 30 min at 4°C, washed twice in PBS containing 1% BSA, incubated 30 min in 200 μ l of conjugate, washed twice in PBS containing 1% BSA, and twice in plain PBS, and fixed in 2.5% glutaraldehyde (Taab Laboratories, Reading, England, EM grade) in 0.10 M cacodylate buffer, pH 7.4, for 2 h at 20°C. The cells were fixed as a pellet by layering the fixative gently over the cells at the bottom of a centrifuge tube. After \approx 10 min, the cell pellet could be raised into the fixative. Cell pellets were washed for 17 h in cacodylate buffer and 20 min in distilled water, postfixed 1 h at 4°C in osmium-ferrocyanide (8), washed, dehydrated in ethanols, and embedded in Epon-araldite (Ladd Research Ind., Burlington, Vt.). Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM300 electron microscope (Philips Electronic Instruments, Inc., Sydney, Australia).

Estimation of Labeling Density. Pancreatic β -cells were photographed in two C57BL/10Sn and one B10.BR/SgSn islet cell preparations. About 50 photographs were taken in each cell preparation and printed at \times 60,000. Similarly, 10 photographs were made of pancreatic duct epithelial cells in the two C57BL/10Sn islet cell preparations. The regions to be photographed were selected at low magnification to show well preserved membrane. Ferritin molecules were counted on the prints and expressed as the number of ferritin molecules per 10-cm length of cell membrane on the prints.

² Abbreviations used in this paper: BSA, bovine serum albumin, PBS, phosphate-buffered saline, PLP, periodate-lysine-paraformaldehyde.

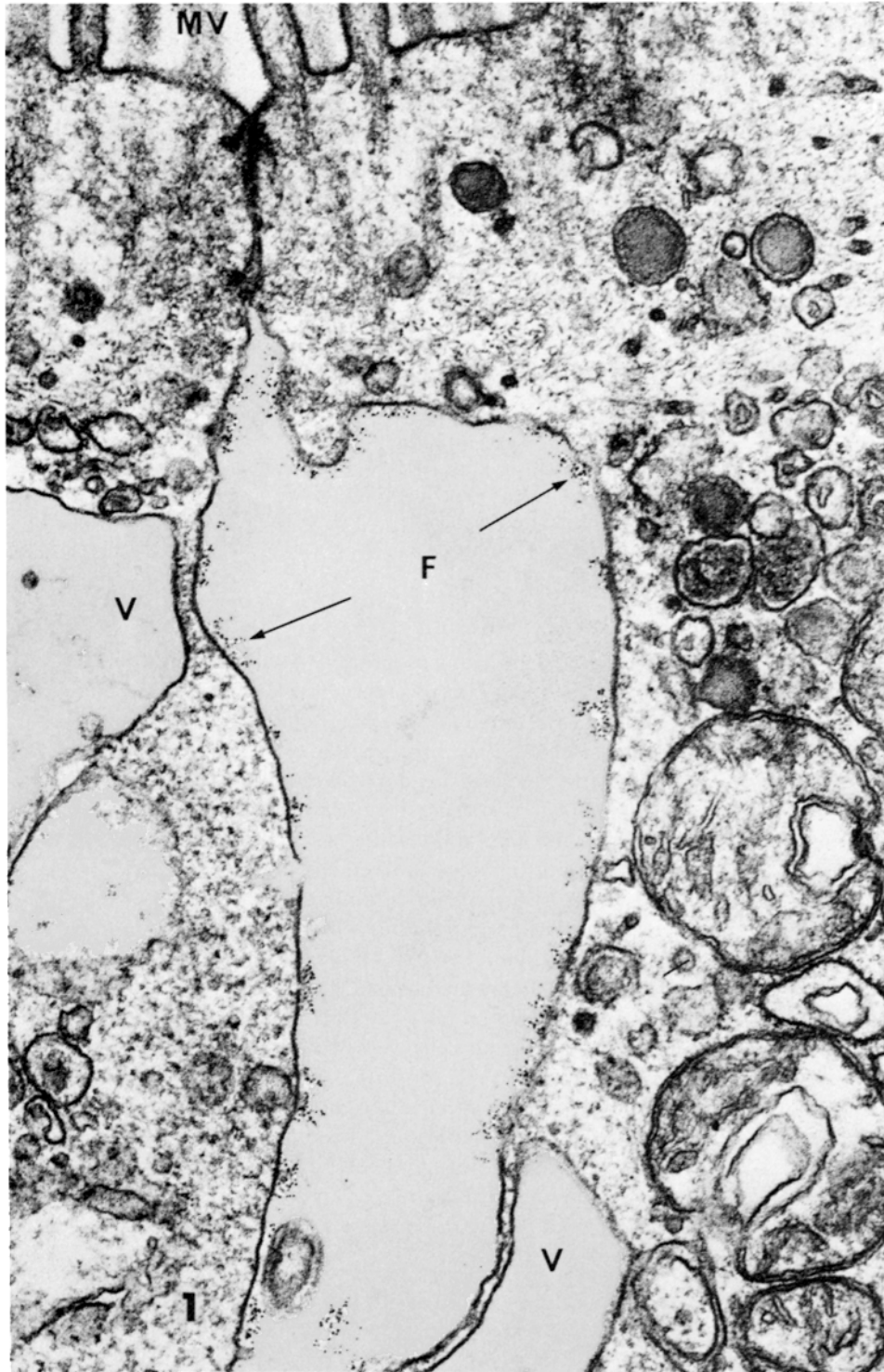


FIG. 1. Pancreatic duct epithelial cells were recognized by their cuboidal shape and microvilli (MV) at the apical end of the cell. Here, two adjacent duct epithelial cells in a C57BL/10Sn islet cell preparation are joined at the junctional complex, but their lateral membranes are well separated. There are several large vacuoles (V) in the cytoplasm. Ferritin-labeled H-2 antigens (F) are present on the lateral cell membranes but not on the apical microvillus membranes. $\times 64,000$.

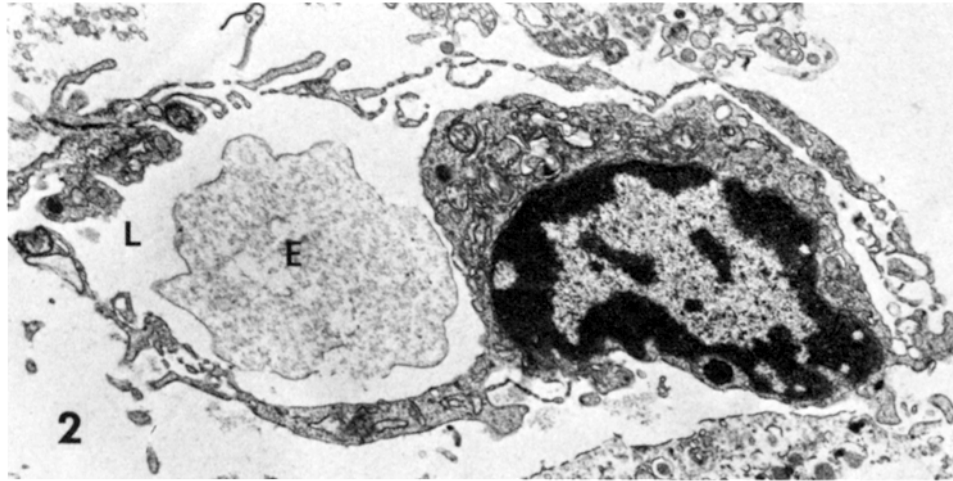


FIG. 2. Capillary endothelial cells in the islet cell preparations showed clearly defined capillary lumens (L) containing an occasional lysed erythrocyte (E) and bounded by fenestrated cell processes. $\times 6,600$.

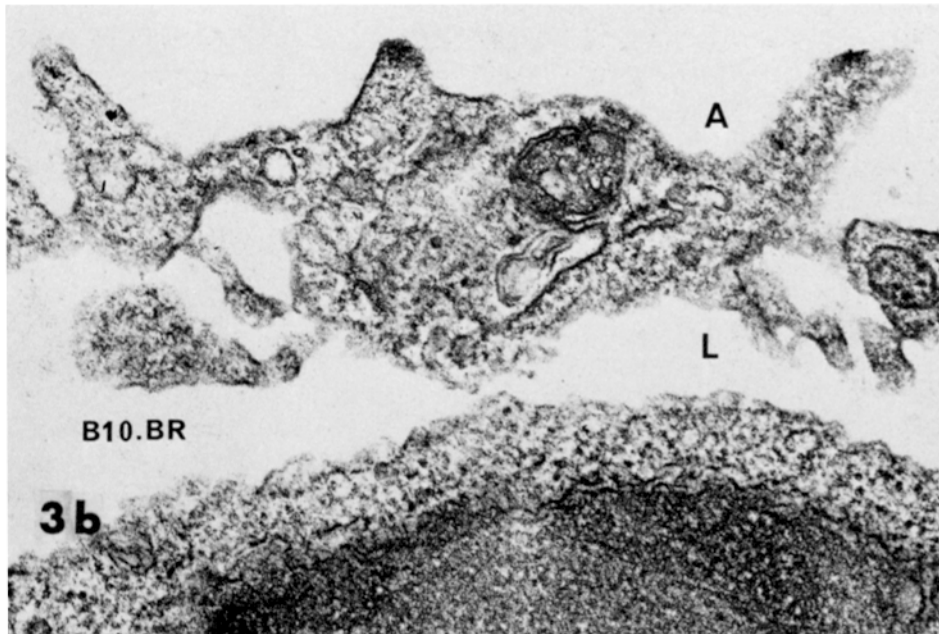
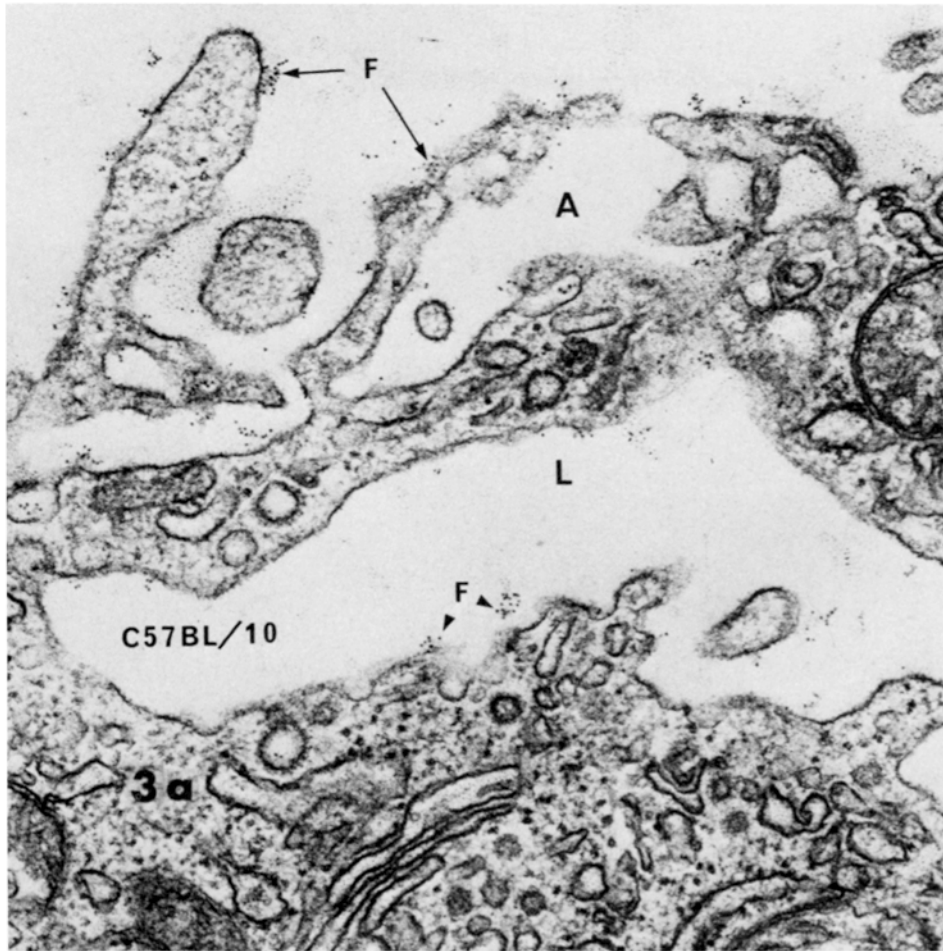
Results

Four cell types were present in significant proportions in cell preparations derived from the isolated islets of Langerhans. These were β -cells, capillary endothelial cells, acinar cells, and pancreatic duct epithelial cells. The nonislet cells were apparently associated with the surface of isolated islets because they cosedimented with the islets during numerous washing steps. The cell preparations contained approximately the same kinds and relative proportions of cells whether the islets were collected by pipetting or by Ficoll-gradient sedimentation. Other cell types, such as α -cells, were either absent or not present in large enough numbers to allow study.

Ferritin-labeled H-2 antigens were present on pancreatic duct cells, acinar cells, and capillary endothelial cells. The corresponding cells in the B10.BR/SgSn control islet cell preparations showed negligible ferritin binding. Pancreatic duct epithelial cells were labeled on lateral and basal membranes, but not on apical membranes (Fig. 1). Endothelial cells were labeled on both luminal and abluminal sides (Figs. 2 and 3). The lateral membranes of acinar cells were labeled (Fig. 4) but their apical membranes were not identified with certainty. In a separate control experiment for the possibility that the collagenase preparation might degrade H-2 antigens, peritoneal macrophages, exposed to 1 mg/ml collagenase for 15 min at 37°C, showed specific labeling of H-2 antigens as dense as on cells not exposed to collagenase.

In contrast to the specific labeling of H-2 antigens on pancreatic duct, acinar, and endothelial cells, no labeling of the antigens could be detected on pancreatic β -cells,

FIG. 3. (a) A capillary endothelial cell in a C57BL/10Sn islet cell preparation. The luminal (L) and abluminal (A) surfaces of the cell membrane are indicated. Ferritin-labeled H-2 antigens (F) are present on both membrane surfaces. The ferritin-labeling density on endothelial cells appeared to be somewhat less than on pancreatic duct epithelial cells. $\times 64,000$. (b) A capillary endothelial cell in a B10.BR/SgSn islet cell preparation that was exposed to all labeling reagents. The luminal (L) and abluminal (A) surfaces of the cell membrane are indicated. Nonspecific ferritin binding is negligible. $\times 64,000$.



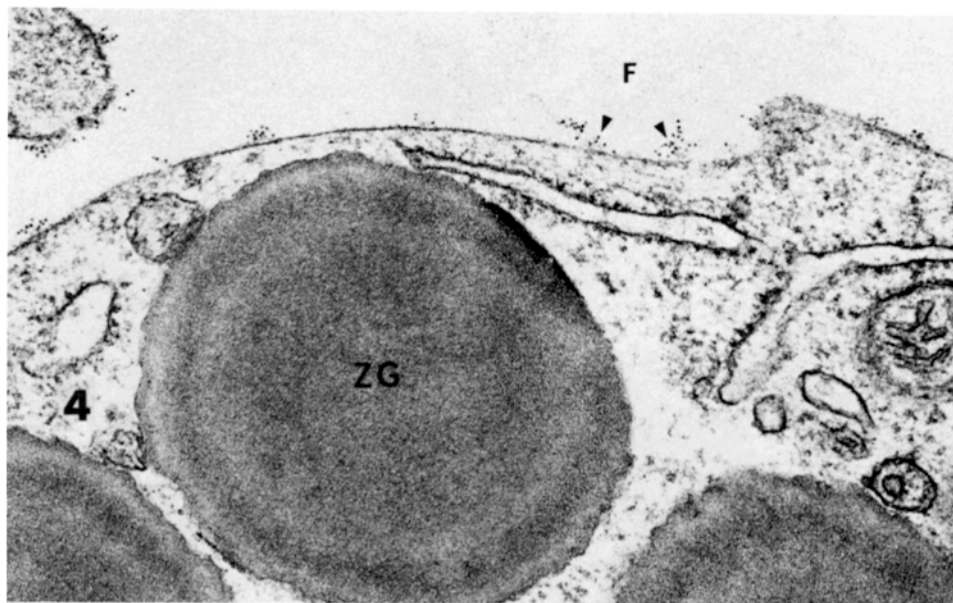


FIG. 4. This shows a portion of a pancreatic acinar cell containing characteristic zymogen granules (ZG) in a C57BL/10Sn islet cell preparation. Ferritin-labeled H-2 antigens (F) are present on the lateral cell membrane. The labeling density is less than on the lateral membranes of pancreatic duct epithelial cells in the same preparation. $\times 64,000$.

with or without prefixation of the islets (Figs. 5 and 6). The average number of ferritin molecules per 10-cm length of β -cell membrane on prints was 0.79, and 1.04 in two C57BL/10Sn preparations and 0.73 in one B10.BR/SgSn preparation. There were an average of 158 ferritin molecules bound per 10-cm length of C57BL/10Sn pancreatic duct cell lateral membrane. Thus, H-2 antigens were either absent from β -cells or they were present in such small amounts that immunoferritin labeling did not distinguish them from nonspecific ferritin binding.

Discussion

The dissociation of β -cells from the pancreas involved digestion with collagenase and trypsin, and fixation in PLP. It is not likely that these procedures destroyed H-2 antigens on β -cells, because the antigens were still present on the other cell types in the same preparations. Additionally, we found that collagenase had no effect on the H-2 antigens of peritoneal macrophages, and a previous study has shown that trypsin and PLP fixation are also without effect on the antigens (2).

Pancreatic β -cells are not unique in lacking demonstrable H-2 antigens. We have examined a total of fifteen different kinds of epithelial cells from various mouse organs, and six of these cell types did not express H-2 antigens (1).¹ Although the epithelial cells and, perhaps, other parenchymal cells of tissues appear to be variable in their expression of H-2 antigens, the presence of these molecules on capillary endothelial cells from islets of Langerhans suggests that vascular endothelium may provide a common source of the antigens for all mouse tissues. In this respect, vascular endothelium may be similar to passenger leukocytes (9-11).



FIG. 5. Pancreatic β -cells were present in the islet cell preparations as single cells or small aggregates of several cells. They were readily identified by their characteristic secretory granules (SG), in which the central dense material was separated from the limiting membrane by an apparently empty space. $\times 6,600$.

The pattern of expression of the major histocompatibility complex on pancreatic cells has interesting implications for the control of diabetes mellitus by transplantation. Allografts of whole islets of Langerhans are known to be rejected and the recipients become sensitized (12). This is consistent with our observation of antigenic cell types in the islets, including capillary endothelium and, probably, acinar cells and duct cells associated with the islets. The β -cells, although they appear to be nonantigenic, may be destroyed nonspecifically during the host attack on the adjacent antigenic cells. It would be of interest to study the fate of β -cells in allografts of single-cell

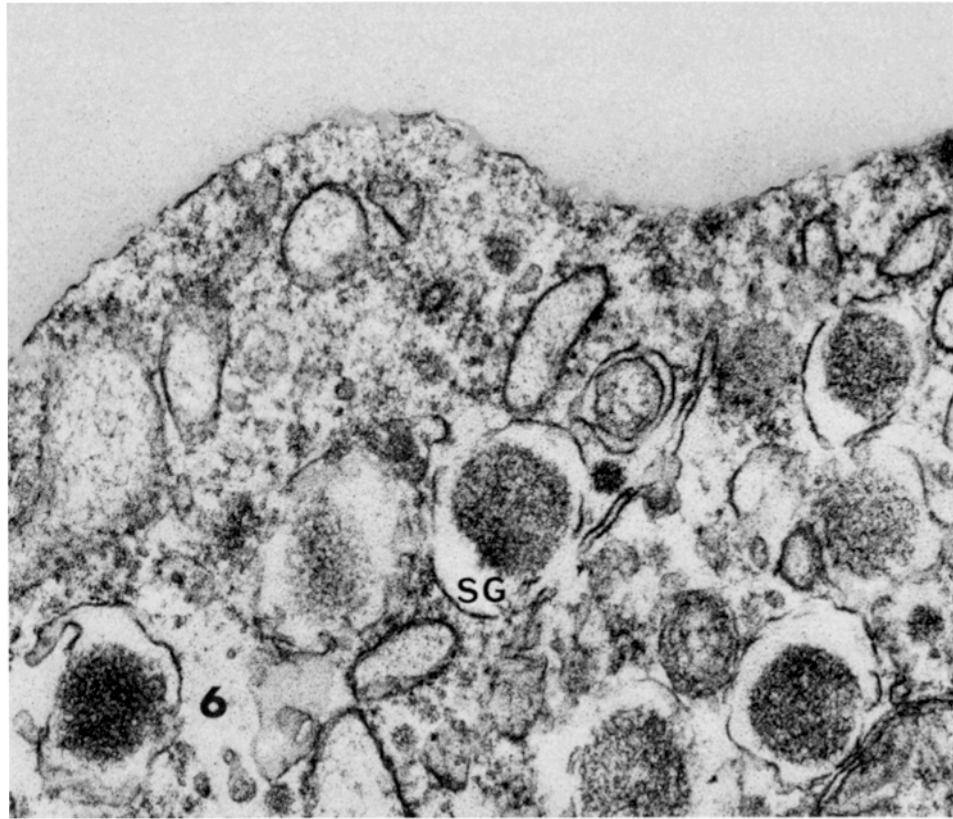


FIG. 6. A pancreatic β -cell showing characteristic granules (SG) in a C57BL/10Sn islet cell preparation exposed to all labeling reagents. There is no ferritin labeling on the cell surface membrane, indicating that H-2 antigens are not present. $\times 64,000$.

suspensions derived from islets. The transplantation of β -cells in suspension might succeed because endocrine cells are uniquely suited to function as single cells in a well vascularized graft site. In this situation, the β -cells would be separated from the antigenic cells and the host might be able to destroy the antigenic cells without harming the β -cells.

Summary

Islets of Langerhans were isolated from mouse pancreases and fixed in periodate-lysine-paraformaldehyde. The fixed islets were then dissociated with trypsin and EDTA to yield cell suspensions that contained mainly four cell types: β -cells, capillary endothelial cells, acinar cells, and pancreatic duct epithelial cells. The nonislet cells were probably associated with the surface of the isolated islets. The H-2 antigens of the dissociated pancreatic cells were labeled with an immunoferritin technique.

Pancreatic duct epithelial cells showed specific ferritin labeling on their lateral cell membranes but not on apical microvillus membranes. Acinar cells were also labeled on lateral membranes, and the capillary endothelial cells were labeled on both the luminal and abluminal aspects of their surface membranes. In contrast, pancreatic

β -cells were unlabeled. The number of ferritin molecules per unit length of β -cell membrane was essentially the same on cells from the antigenic strain and the congeneric control strain, and was about 200-fold less than on the labeled pancreatic duct epithelial cell lateral membranes. Pancreatic β -cells are therefore one of six known epithelial cell types on which H-2 antigens can not be detected by immunoferritin labeling. The apparent absence of H-2 antigens from these cells suggests a study of the viability of β -cells in allografts of dissociated islet cells, in which the β -cells would not be in contact with antigenic cells. Such studies might lead to a new approach to the control of diabetes mellitus by transplantation.

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