# Original Articles

# Allotransplantation of Culture-Isolated Neonatal Rat Islet Tissue

## Absence of MHC Class II Positive Antigen-Presenting Cells in Nonimmunogenic Islets

ORION D. HEGRE, ROBERT J. KETCHUM, HEINZ POPIELA, CINDY R. EIDE, R. MARK MELOCHE, JANET R. SERIE, AND WAYNE V. MOORE

**When highly purified neonatal rat islet tissue, derived after 10 days in vitro, was allografted, it was found to be nonimmunogenic or weakly immunogenic. In contrast, nonislet pancreatic components, derived from the same culture system, transplanted with highly purified islet tissue resulted in rejection in 88% of cases. Extension of the culture period did not result in reduced immunogenicity of the nonislet material. Immunostaining of islet or nonislet tissue from the culture system failed to demonstrate major histocompatibility complex (MHC) class II positive cells in the islet tissue, whereas the presence of MHC class II staining cells in the nonislet components was clearly demonstrable. These results demonstrate that the islet tissue obtained by culture isolation is free of cells capable of stimulating an allogeneic immune response and are consistent with the hypothesis that the absence of MHC class II positive antigen-presenting cells reduces the immunogenicity of the tissue and enhances the survival of allogeneic grafts. _Diabetes_ 38:146–51, 1989**

N umerous studies have shown that the in vitro maintenance of various tissues before transplantation can effectively alter the tissues' immunogenicity (1,2). This is believed to be due to elimination or reduction in the number of passenger leukocytes within the tissue during culture (1,3). We recently reported the long-term survival of cultured neonatal rat islets across six different combined major and minor histocompatibility barriers with an in vitro method for islet purification

in which recipient animals were not immunosuppressed (4,5). The technique did not employ elevated oxygen levels or other leukotoxic treatments. This marked increase in graft survival is presumably due to the elimination or incapacitation of antigen-presenting cells (APCs) from the islet compartment during culture. The following experiments were designed to determine if the in vitro method of islet isolation and culture eliminates functional APCs from only the islet compartment or from the entire culture system.

## MATERIALS AND METHODS

**Animals.** Donor animals were 4- to 5-day-old male and female Fischer-344 (F344) of major histocompatibility complex haplotype Rt1$^l$ rats and Wistar-Furth (WF) of MHC haplotype Rt1$^u$ rats. Recipients were normal adult male F344 and WF rats free of any immunosuppressive therapy.

**Islet isolation and culture.** The culture-isolation method has been previously described (6). Pancreases from one litter, normally ~10 pups, were dissected free and minced. The minced tissue was then cultured in Ham's F-12 (Gibco, Grand Island, NY) supplemented with 5% fetal calf serum (Hyclone, Logan, UT) in 5% $CO_2$/95% air at 37°C. Medium was renewed on day 4 of culture. After 7 days, material was recovered from the dish by agitation followed by centrifugation. The pellet was replated in 100-mm plastic dishes and incubated in fresh medium under the same conditions for an additional 24 h. This material was then removed by agitation, centrifuged, and replated to 100-mm plastic dishes. Islets were then handpicked free of nonendocrine components based on morphological characteristics. Incubation of islets and nonislet material was continued separately for varying periods of subculture under the same conditions, with the exception of the use of 25% horse serum in place of fetal calf serum. Subculture time ranged from 2 to 20 days. An antibiotic/antimycotic (Gibco) was added to all media at a 1% concentration.

**Transplantation.** Transplantation of cultured material was conducted as previously described at the kidney subcapsular site (7). Donor tissue was derived from eight litters,

each cultured separately as described above. Approximately 200 μg of islet or nonislet tissue was transplanted via micropipette to each animal. Islet mass was determined by computer-assisted morphometric analysis as previously described (5). The average islet mass was determined by this method to be 0.8 μg. Therefore, each recipient received ~250 highly purified islets or an equivalent mass of cultured material consisting primarily of nonislet tissue with a small number of purified islets added at the time of transplantation. The islets were added to provide a histological marker of the site of the graft. A further series of 20 animals received allogeneic islet transplants. Six animals were left untreated for an additional 21 days as control animals. The remaining 14 animals were challenged by a simultaneous transplantation of nonislet pancreatic tissue at the opposite pole of the kidney. Of these 14 animals, 6 received viable nonislet tissue, 6 received twice-frozen and thawed nonislet tissue, and 2 received fixed (24 h in 24 mM HCl in 95% ethanol) nonislet tissue. Grafts were excised 21 days after transplantation and subsequently evaluated histologically.

**Assessment of rejection.** At intervals after transplantation, graft areas were recovered and examined histologically. Tissue blocks were fixed in Bouin's solution and prepared for routine paraffin histology. Adjacent sections at 40-μm intervals throughout the entire graft area were stained with either hematoxylin-eosin or aldehyde fuchsin-Ponceau de xylidine (AF). The immune response by the host was assessed by the histological appearance of the graft as follows: 0, histologically intact islets with AF+ β-cells and/or viable ductal elements with no accumulation of mononuclear cells (MNCs) in the graft area; 1+, intact islet or nonislet tissue with minor MNC infiltration; 2+, intact islets or ductal elements with extensive MNC infiltration; 3+, damaged islet endocrine or ductal epithelial cells, AF+ scar areas, giant foreign body cells, or massive MNC infiltration; and 4+, primarily scar areas with some MNCs still present and little or no graft tissue remaining.

**Immunohistochemistry.** Islets or nonislet pancreatic components derived from the culture-isolation system were snap frozen in liquid nitrogen, sectioned at a thickness of 5 μm, mounted, and stained with the avidin-biotin-peroxidase technique (8,9; Vector, Burlingame, CA). The mounted sections were air dried at 37°C for 10 min. Sections were then rehydrated in phosphate-buffered saline (PBS) and subsequently incubated with a mouse anti-rat MHC class II antibody for 60 min at room temperature (OX6 from Seralab, Accurate Chemical, Westbury, NY). This was followed by incubation with biotinylated horse anti-mouse antibody for 30 min at room temperature and then by incubation with avidin-biotin complex (ABC), also for 30 min at room temperature. Diaminobenzidine (DAB; 0.02% solution) was used as the chromagen. All sections were lightly counterstained with hematoxylin. Islets were also immunostained for insulin and glucagon with guinea pig anti-pork insulin (10) and rabbit anti-human glucagon (11), respectively, for 18 h at 4°C. The sections were treated with either biotinylated horse anti–guinea pig antibody or biotinylated horse anti-rabbit antibody, as appropriate, for 30 min at room temperature. These sections were then treated with ABC and DAB as above. All reagents were made with PBS.

**Quantification of immunohistochemical staining.** Cross-sections of culture-isolated islets or nonislet-cultured pancreatic fragments were examined and categorized as those either staining or not staining with the OX6 antibody. Individual fragments displaying staining were then evaluated by counting total nuclei within the cell aggregate, then counting nuclei of cells demonstrating staining. Areas appearing acellular in nature (i.e., nuclei not visible) were not considered in this method of quantification.

## RESULTS

Highly purified, culture-derived islets from either F344 or WF rats were allotransplantable (Table 1). No immune response occurred in WF recipients of pure F344 islet grafts. AF+ β-cells were present at all graft sites. No mononuclear cell accumulations were observed (Fig. 1). The same results were obtained in 50% of F344 recipients of WF islets. In the remaining grafts, analyzed at >40 days after transplantation, morphologically intact islets remained at the graft site with no evidence of intraislet invasion by MNCs or graft destruction. However, peripheral MNC infiltrates were clearly associated with the transplanted islets.

In contrast to the success obtained when highly purified islets were allotransplanted, inclusion of nonislet pancreatic components from culture compromised survival of the islet tissue and resulted in rejection of both the islet and nonislet components in most cases (Table 2 and Fig. 2). Prolonged maintenance of nonislet material in subculture did not reduce the immunogenicity of the tissue (Table 2). This nonislet material is heterogeneous, consisting of various cell types, including ductal epithelium, stroma, and endocrine cells.

Simultaneous challenge of an islet allograft with viable nonislet pancreatic explants from culture resulted in the rejection of both the nonislet and islet grafts in 5 of 6 cases (Fig. 3). When nonviable nonislet (i.e., frozen/thawed or fixed) tissue was used to challenge, no rejection of the islet graft occurred (Table 3).

Immunostaining of highly purified culture-isolated islets with anti-insulin and anti-glucagon antibodies demonstrated the distribution of cells containing these hormones in a pat-

TABLE 1
Allotransplantation of highly purified islet tissue

| Donor strain | Recipient strain | n | Days in culture | Term in situ (days) | Histological evaluation of graft rejection | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0 | +1 | +2 | +3 | +4 |
| Fischer-344 | Wistar-Furth | 10 | 10 | 40 | 10 | | | | |
| Wistar-Furth | Fischer-344 | 10 | 10 | 40 | 5 | | 5 | | |

See MATERIALS AND METHODS for descriptions of graft rejection responses.
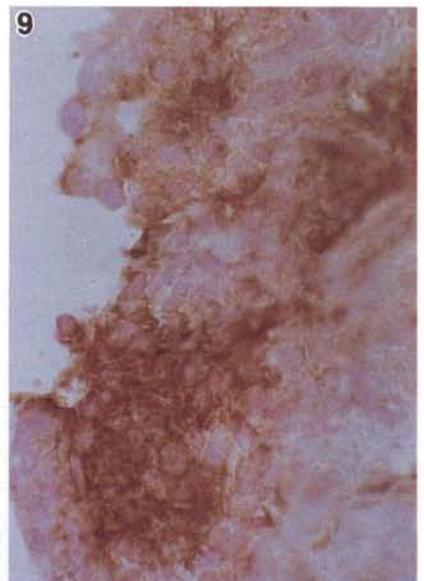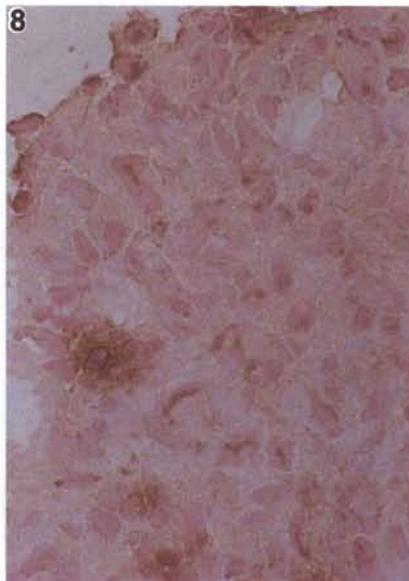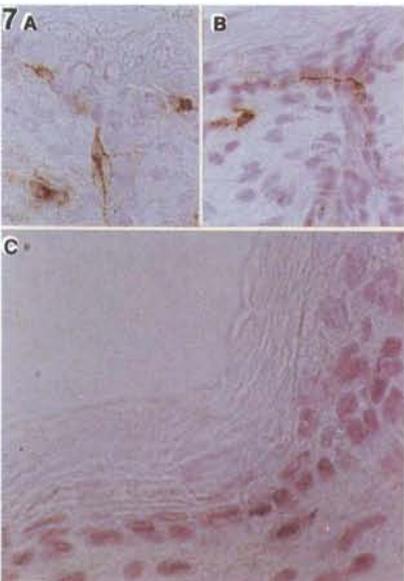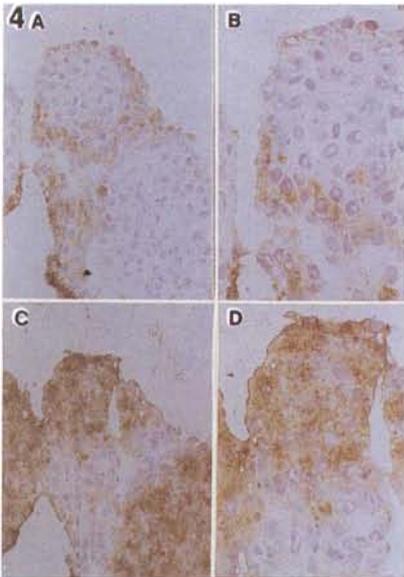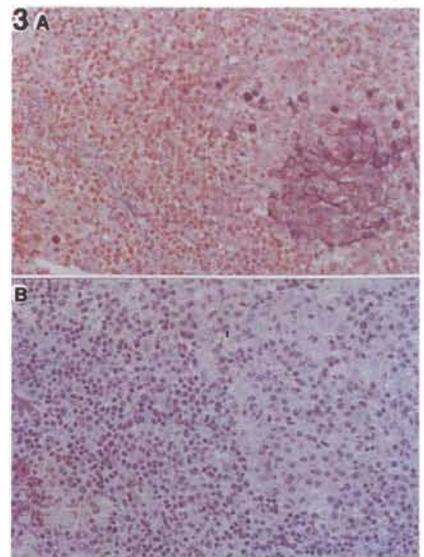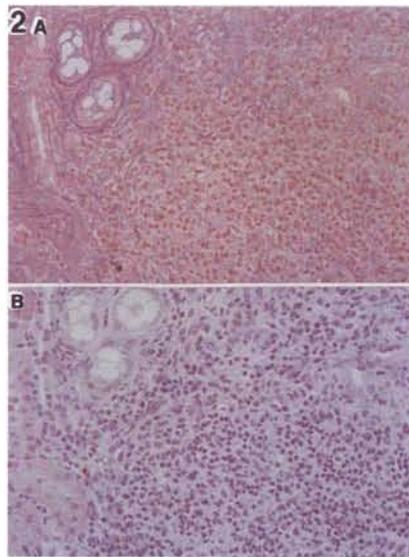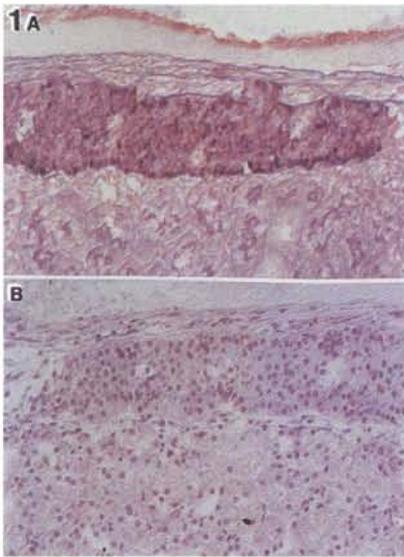
TABLE 2
Allotransplantation of nonpurified islet tissue

| Parameter | n | Term in situ (days) | Histological evaluation of graft rejection | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | +1 | +2 | +3 | +4 |
| F344 to WF | 7 | 10–20 | | | | 1 | 6 |
| WF to F344 | 7 | 10–20 | | | | 1 | 6 |
| >20 days in culture* | 7 | 10–20 | | | | 1 | 6 |
| 10 days in culture* | 7 | 10–20 | | | | 1 | 6 |
| <10 days in situ* | 6 | <10 | 2 | | 4 | | |

F344, Fischer-344 rats; WF, Wistar-Furth rats. See MATERIALS AND METHODS for descriptions of graft rejection responses.
*F344 donor, WF recipient.

TABLE 3
Immunological challenge of culture-purified islet allografts

| Islet allograft challenged with | n | Term in situ (days) | Histological evaluation of graft rejection | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | +1 | +2 | +3 | +4 |
| Unchallenged | 6 | 21 | 6 | | | | |
| Viable nonislet tissue | 6 | 21 | 1 | | | | 5 |
| Frozen nonislet tissue | 6 | 21 | 4 | 1 | 1 | | |
| Fixed nonislet tissue | 2 | 21 | 2 | | | | |

All grafts were Fischer-344 islets to Wistar-Furth host. See MATERIALS AND METHODS for descriptions of graft rejection responses.

tern consistent with that of the typical mantle islet (Fig. 4). Subsequent staining of the islet tissue with the anti-rat MHC class II antibody OX6 failed to demonstrate the presence of any class II positive cells within these highly purified islets (Figs. 5 and 6). Multiple cross sections of >300 islets have been stained and examined.

Staining of skin sections cut from abdominal skin of F344 neonates demonstrated the effectiveness of this antibody in staining the class II expressing Langerhans cell in the basal layer of the epidermis. Control sections treated identically, with the exception of the primary antibody (OX6), showed that this staining was not due to nonspecific binding of the secondary antibody or ABC, nor to endogenous peroxidase activity in the tissue (Fig. 7).

Staining of the nonislet pancreatic components with the anti-rat MHC class II antibody labeled numerous cells expressing class II proteins at their surface (Figs. 8 and 9). Occasional staining was observed in what appeared to be acellular regions within the nonendocrine cell aggregates. This staining was not considered to represent positive staining of class II at the cell surface and was discounted from quantification. Only cells clearly identifiable, based on general cell morphology and distinguishable nuclei, were included in this initial attempt to quantify class II expression in cultured nonislet pancreatic tissue.

Approximately 40% of the >300 cross sections of nonislet cellular aggregates evaluated displayed positive staining. Of those aggregates demonstrating staining, ~30% of the cells stained positively. This staining represented individual cells scattered throughout nonstaining cells within a cell aggregate (Fig. 8), small groups of a few to several cells within aggregates of nonstaining tissue (Fig. 9), and, in a few cases, large numbers of cells that comprised the majority of some cell aggregates. Overall, the stained tissue represented ~10% of the total nonislet tissue examined.

## DISCUSSION

We have previously shown that islets derived from culture-isolation are transplantable across strain barriers (4,5). The results reported here demonstrate the requirement for pure islet endocrine tissue, free of any contaminating nonendocrine cells, for the successful allogeneic transplantation of neonatal islet tissue. The allotransplants of F344 islets to WF hosts were entirely free of any MNC infiltrate, as were 50% of WF islet allografts to F344 hosts. Although 50% of WF islets allografted to F344 recipients displayed a peripheral lymphocytic accumulation, these grafts were examined at >40 days after transplantation, and intact islet tissue, with little or no evidence of endocrine cell damage, was observed at the graft site. We and others have reported a similar phe-

FIGS. 1–9.
*1:* Adjacent sections of highly purified culture-isolated Fischer-344 neonatal islets transplanted allogeneically to kidney capsule of Wistar-Furth host, demonstrating viable graft tissue with no evidence of immune response. Stained with aldehyde fuchsin (A) and hematoxylin and eosin (B). ×140.
*2:* Adjacent sections of cultured Fischer-344 nonislet pancreatic tissue transplanted to kidney subcapsule of Wistar-Furth host, showing extensive infiltration of lymphocytes with graft destruction nearly complete. Some ductal elements remain, visible at *upper left.* Stained with aldehyde fuchsin (A) and hematoxylin and eosin (B). ×140.
*3:* Adjacent sections of highly purified culture-isolated Fischer-344 neonatal islets transplanted to kidney capsule of Wistar-Furth host simultaneously challenged with cultured Fischer-344 nonislet pancreatic tissue. Graft shows definite evidence of islet destruction with extreme mononuclear cell infiltrate. Stained with aldehyde fuchsin (A) and hematoxylin and eosin (B). ×140.
*4:* Near-adjacent frozen sections of culture-isolated islets immunostained for glucagon (A and B) and insulin (C and D). Although some fusion with neighboring islets occurred during processing, characteristic distribution of these 2 islet hormones, central staining for insulin and peripheral staining for glucagon, is observed. A and C, ×200; B and D, ×350.
*5:* Frozen section of culture-isolated islets immunostained with OX6 and lightly counterstained with hematoxylin. No OX6 positivity is visible, indicating no class II positive cells present in preparation. ×350.
*6:* High-power view of frozen section of culture-isolated islets immunostained with OX6 and lightly counterstained with hematoxylin. No class II positive cells are evident. ×950.
*7:* Control tissue demonstrating specificity of binding by major histocompatibility complex class II specific 1° antibody OX6 (A and B) and lack of nonspecific binding by 2° antibody (C). Langerhans cells in abdominal skin of 4- to 5-day-old neonates showing dendritic morphology typical of these cells. A and B, ×400; C, ×500.
*8:* High-power view of frozen section of cultured nonislet pancreatic tissue demonstrating presence of scattered individual class II positive cells within cellular aggregate. Immunostained with OX6 and lightly counterstained with hematoxylin. ×950.
*9:* High-power view of frozen section of cultured nonislet pancreatic tissue demonstrating presence of clusters of class II positive stained cells within cellular aggregate. Immunostained with OX6 and lightly counterstained with hematoxylin. ×950.

nomenon of peripheral MNC infiltrate surrounding xeno-grafted tissue (7,12). The grafted tissue remains functional, although surrounded by cells identified as belonging to the suppressor/cytotoxic subset (13). Due to the lack of cyto-toxic effects on the graft, it was concluded that these cells represented a population of suppressor cells, rather than cytotoxic cells, encircling the xenografted tissue. Although not xenogeneic, WF islets in an F344 host might possibly be promoting a similar type of response. Further studies are in progress to better define this observed chronic immune in-filtrate.

The class II negative islets used in these studies were culture derived from neonatal pancreas. Inclusion of nonislet material, also derived from culture isolation, which contains class II positive cells, results in islet allograft rejection. Re-cent studies in the mouse have shown that islets, obtained by collagenase digestion and transplanted to an allogeneic host, demonstrate prolonged mean survival times when highly purified of contaminating nonislet tissue (14). Both neonatal and adult islets, isolated by collagenase digestion and highly purified by handpicking, are rapidly rejected by the host when allogeneically transplanted (unpublished ob-servations). Thus, by purifying islet tissue from nonislet tis-sue, the immunogenicity of the grafted tissue is decreased. However, islets isolated by collagenase digestion still con-tain the class II positive cells residing within them at the time of their isolation from the pancreas (15) and, therefore, retain some degree of immunogenicity.

During culture, incubation conditions were optimized for general cell survival, and thus, a number of cell types, in-cluding pancreatic ductal and stromal elements and islet endocrine tissue, were maintained during culture. It has been reported elsewhere that in vitro culture conditions are com-patible with class II positive dendritic cell (i.e., APC) survival (16). We also found that viable class II positive APCs are maintained in our culture system and may survive the in vitro period for up to several weeks. They are, however, found only in the nonislet tissue compartment.

Thus, culture isolation yields islets with reduced immu-nogenicity. The cellular purity of the endocrine tissue gen-erated in vitro (6) has been confirmed by immuno-histochemistry. Class II bearing cells are not found within islets derived from culture isolation. These data are con-sistent with the hypothesis that such APCs are required for effective antigen presentation to occur in allotransplanted tissues (1) and that they can provide the costimulator activity needed to activate a specific T-lymphocyte response (17). A similar correlation between the absence of class II positive cells and allotransplantability has been reported for cultured keratinocyte allografts (18).

These results further indicate that although culture isola-tion alters the immunogenicity of the islets, it does not alter the antigenicity of the endocrine cells, because culture-de-rived endocrine tissue can serve as a target for an immune response and will be rejected when effective antigen pre-sentation occurs. This occurs when impure preparations de-rived from the culture system, containing class II bearing cells, are allotransplanted with the purified islets or are used to challenge islet allografts. These class II bearing cellular

preparations must contain viable tissue for an allogeneic immune response to be generated.

Recently, we have shown such cells to be present within the islet compartment by 4–5 days postnatally (19). This indicates that the absence of APCs from culture-derived is-lets is not due to their absence from the islet at the time of explantation. Culture conditions may promote the selective loss of APCs from the islet compartment by a mechanism such as migration onto the culture substratum. This possi-bility seems unlikely, however, because this would represent a loss of APCs by islet tissue only, whereas nonislet tissue retains its population of APCs without loss due to such se-lective migration. Islets derived from culture isolation may arise primarily by in vitro islet neogenesis from ductal epi-thelium during culture. In the absence of a vascular network and with no bone marrow source, these islets generated during the culture process could be expected to be devoid of APCs. These possibilities are being examined.

## REFERENCES

1. Lafferty KJ, Prowse SE, Simeonovic CJ, Warren H: Immunobiology of tissue transplantation: a return to the passenger leukocyte concept. *Annu Rev Immunol* 1:143–73, 1983
2. Lacy PE, Davie JM, Finke EH: Prolongation of islet allograft survival fol-lowing in vitro culture (24°C) and a single injection of ALS. *Science* 204:312–13, 1979
3. Lechler RI, Bachelor JR: Restoration of immunogenicity to passenger cell–depleted kidney allografts by the addition of donor strain dendritic cells. *J Exp Med* 155:31–41, 1982
4. Serie JR, Hegre OD, Eide CR, Weinhaus AJ, Marshall S: The successful allotransplantation of neonatal rat islets across multiple combined major and minor histocompatibility barriers. *Transplantation* 44:739–41, 1987
5. Hegre OD, Hickey GE, Marshall S, Serie JR: Modification of allograft immunogenicity in perinatal islets isolated and purified in vitro. *Trans-plantation* 37:227–33, 1984
6. Hegre OD, Marshall S, Schulte BA, Hickey GE, Williams F, Sorenson RL, Serie JR: Nonenzymic in vitro isolation of perinatal islets of Langerhans. *In Vitro* 19:611–20, 1983
7. Serie JR, Hickey GE, Schmitt RV, Hegre OD: Prolongation of culture-isolated neonatal islet xenografts without immunosuppression. *Trans-plantation* 36:6–11, 1983
8. Hsu SM, Raine L, Fanger H: A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Pathol* 75:734–38, 1981
9. Hsu SM, Raine L, Fanger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. *J Histochem Cytochem* 29:577–80, 1981
10. Morgan CR, Lazarow A: Immunoassay of insulin: two antibody system: plasma levels of normal, subdiabetic and diabetic rats. *Diabetes* 12:115–26, 1963
11. McEvoy RC, Madsen KL, Elde RP: Stimulation of anti-glucagon antibodies in rabbits. *Metab Res* 9:272–74, 1977
12. Lacy PE, Finke EH, Janney CG, Davie JM: Prolongation of islet xenograft survival by in vitro culture of rat megaislets in 95% $O_2$. *Transplantation* 33:588–92, 1982
13. Janney CG, Davie JM, Lacy PE, Finke EH: Characterization of lympho-cytes from rejected and nonrejected islet xenografts. *Transplantation* 33:585–87, 1982
14. Gotch M, Maki T, Satomi S, Porter J, Monaco AP: Immunological char-

acteristics of purified pancreatic islet grafts. *Transplantation* 42:387–90, 1986

15. Faustman D, Hauptfeld V, Lacy P, Davie J: Prolongation of murine islet allograft survival by pretreatment of islets with antibody directed to Ia determinants. *Proc Natl Acad Sci USA* 78:5156–59, 1981

16. Steinman RM, Kaplan G, Witmer MD, Cohn ZA: Identification of a novel cell type in peripheral lymphoid organs of mice. *J Exp Med* 149:1–16, 1979

17. Lafferty KJ, Woolnaugh JA: The origin and mechanism of allograft reactions. *Immunol Rev* 35:231–62, 1977

18. Hammond EJ, Ng RLH, Stanley MA, Munro AJ: Prolonged survival of cultured keratinocyte allografts in the non-immunosuppressed mouse. *Transplantation* 44:106–12, 1987

19. Meloche M, Ketchum RJ, Serie JR, Sutherland DER, Hegre OD: Elimination of Ia-bearing cells by in vitro isolation and culture of neonatal rat pancreatic islets. *Transplantation* 46:614–15, 1988