Yield and Viability of Human Fetal Isletlike Cell Clusters Obtained After Different Types of Abortion

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Human fetal pancreas is a potential source of insulin-secreting tissue for clinical transplantation. However, collection and preservation of sufficient amounts of viable tissue are major problems. We have studied isletlike cell clusters (ICCs) derived from pancreases of 180 human fetuses. The fetal glands were obtained after abortions of different types: 18 by hysterotomy (HY; gestational age 19.6 ± 2.1 wk, mean ± SD), 73 by prostaglandin induction (PG; 18.2 ± 2.3 wk), and 89 by dilation and extraction (DE; 14.5 ± 1.3 wk). After collagenase digestion and culture in medium (RPMI-1640) supplemented with 10% human serum, numerous free-floating ICCs were formed from a single pancreas. The ICCs contained randomly scattered insulin- and glucagon-containing cells, often in contact with ductlike structures. However, the majority of the cells were not positive for either of the hormones. The yield of ICCs (median and range) was 725 (150–1260) after HY, 150 (0–1060) after PG ($P < .001$ vs. HY), and 400 (0–2000) after DE ($P < .001$ vs. HY). Insulin and glucagon levels in the culture medium declined rapidly during the first 7 days, but then remained at constant levels for up to 31 days, despite a simultaneous decrease in the number of ICCs down to approximately one-fifth of the original. Responsiveness of insulin release to 10 mM theophylline plus 20 mM glucose was studied after 1–5 days in culture. The mean ± SE response was 11.5 ± 2.1-fold ($n = 11$) after HY, 5.6 ± 0.7-fold ($n = 25$) after PG ($P < .001$ vs. HY), and 6.0 ± 0.6-fold ($n = 16$) after DE ($P < .05$ vs. HY). In conclusion, the yield and viability of ICCs were clearly better if prostaglandin had not been used. ICCs appear to be a useful tool for studies of human fetal endocrine pancreas. Their suitability for transplantation remains to be studied.

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Identification and Manipulation of Human Islet Alloimmunogenicity: Morphologic and Functional In Vitro Studies With Various Islet Preparations

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Successful human pancreatic islet transplantation will depend on a better knowledge of the cells that contribute to allograft immunogenicity but not to the desired long-term graft function. This knowledge will provide the basis for manipulative procedures to reduce graft immunogenicity. Therefore, immunofluorescence studies and mixed-lymphocyte islet culture (MLIC) tests were performed with cryopreserved crude islet preparations and various HLA and cell-type–specific monoclonal antibodies to *1*) locate the potentially immunogenic cells and *2*) demonstrate their functional relevance before and after inhibition with antibodies. Microscopic evaluation of exocrine and endocrine tissue components of crude islet preparations was greatly facilitated by the dithizone staining technique. The following results were obtained. With dithizone counterstaining, the vast majority of MHC class II–positive cells were found to be located in the exocrine tissue portion; these cells strongly expressed HLA-DR/DP but rarely HLA-DQ. By contrast (and in contrast to rodent models), the endocrine tissue portion, i.e., the pure islets, contained only a few very weakly stained HLA-DR/DP–positive cells. Importantly (and in contrast to our own experience with rat islets), endocrine β-cells were always HLA-DR/DP/DQ negative. Crude islet preparations acted as powerful stimulators in allogeneic MLIC. By contrast, the allogeneic in vitro response was markedly reduced when using relatively clean, handpicked stimulator islets. Preincubation of the crude islet preparations with HLA-DR/DP antibodies also significantly diminished the allogeneic immune response. According to both morphologic and functional in vitro data, the exocrine tissue portion of a crude islet preparation predominantly contributes to islet-graft immunogenicity. Thus, it is suggested that further purification steps before transplantation are required. As long as purification procedures do not yield optimal results, islet preparation pretreatment with HLA-DR/DP antibodies may serve as a valuable substitute.

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