Reduced Immunogenicity of First-Trimester Human Fetal Pancreas

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OBJECTIVE—The use of human fetal pancreatic tissue may provide a potential source of transplantable β-cells as a therapy for type 1 diabetes. Human fetal pancreas has a remarkable capacity to grow and differentiate in vivo and has been shown to reverse diabetes in rodents. However, it is known that human fetal pancreas obtained from the second trimester of gestation is immunogenic and is rejected after transplantation. Tissue obtained from earlier stages might prove to be immune privileged, as has been shown for other tissues.

RESEARCH DESIGN AND METHODS—In this study, we determined the immunogenicity of human fetal pancreatic tissue obtained from the first trimester of gestation in a humanized mouse model. A microarray study of immunoregulatory gene expression in first- and second-trimester human fetal pancreas was also undertaken.

RESULTS—The analysis of transplanted human fetal pancreata revealed a significantly decreased immunogenicity of the first-trimester tissue. The first-trimester grafts showed only limited cellular infiltration and contained numerous insulin-positive cells, whereas second-trimester tissue was completely infiltrated and rejected. Furthermore an analysis of immunoregulatory genes expressed in first- and second-trimester human fetal pancreas by microarray demonstrated the upregulation of several key immunoregulatory genes in the second-trimester tissue. This might account for the reduced immunogenicity of the younger tissue.

CONCLUSIONS—Our results provide the first indication that the use of first-trimester human fetal pancreas for transplantation might increase the survival of the grafts and might decrease the requirement for immunosuppressive drugs. ***Diabetes*** **57:627–634, 2008**

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APC, allophycocyanin; BGL, blood glucose level; cRNA, complimentary RNA; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; H-E, hematoxylin-eosin; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; PE, phycoethrin; RMA, robust multichip analysis; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; UNSW, University of New South Wales.

See accompanying commentary, p. 525.

Type 1 diabetes results from the autoimmune destruction of insulin-producing (β) cells in the pancreas. Replacement of the destroyed β-cells represents the ideal treatment of this disorder. Transplantation of either whole pancreas or islets as a therapy, however, is limited because of the scarce supply of organs and the lifelong need to take immunosuppressive drugs to prevent transplant rejection.

Human fetal pancreas is discussed as a source of transplantable β-cells because it has a much greater proliferative capacity than adult tissue (1,2). Furthermore, after transplantation into diabetic immunoincompetent rodents, the tissue consistently differentiates into a functionally mature graft (2–6) containing β-cells and other endocrine cells (7) and is able to normalize blood glucose levels (BGLs) in diabetic rodents (2,3,5,6). Another advantage of transplanting fetal tissue is its possible immune privileged status, as has been demonstrated for other fetal tissue transplants (8–11). It is known, however, that second-trimester (14–26 weeks of gestation) human fetal pancreas is immunogenic, as demonstrated by its ability to stimulate the proliferation of lymphocytes in a mixed lymphocyte culture (12). When transplanted into a humanized SCID mouse reconstituted with human immune cells, 19- to 21-week-old human fetal pancreata were rejected (13). However, the immunogenicity of human fetal pancreas obtained from the first trimester (up to 13 weeks of gestation) has not been reported so far.

Dekel et al. (14,15) demonstrated that first-trimester human fetal kidneys are less immunogenic than adult and second-trimester kidneys. Transplantation of adult renal grafts and human fetal kidney at 14 weeks gestation into humanized mice resulted in the rejection of these tissues. Kidneys obtained between 7 and 8 weeks gestation showed no signs of rejection. The ability of first-trimester human fetal pancreas obtained between 6 and 9 weeks gestation to grow and mature in vivo and eventually achieve normoglycemia in diabetic mice has been demonstrated by Castaing et al. (2). However, this study did not examine the immunogenicity of the tissue. Reduced immunogenicity has been shown for fetal pig pancreatic tissue in a xenograft model using humanized mice. Only minimal infiltration of human immune cells was observed in day-42 pig embryonic pancreatic grafts, corresponding to early second trimester, whereas day-56 pig pancreatic grafts, corresponding to late second trimester, were extensively infiltrated and rejected (16).

In the present study, we analyzed the immunogenetic potential of first-trimester human fetal pancreas using the human peripheral blood mononuclear cell (PBMC) reconstituted humanized mouse to examine allograft rejection (11,17,18). We also examined differences in the expression

TABLE 1
Characteristics of primers for real-time RT-PCR

Primer (name)	Accession number*	Primer pair sequence (sense/antisense)	Location (nucleotide)	Fragment size (bp)
HLA-A	NM_002116.5	5′-TGTCCCTCACAGCTTGTAAAG-3′	1073–1093	150
		5′-ATTATGCCTACACGAACACAG-3′	1222–1202	
HLA-B	NM_005514.5	5′-CATGACCAGTACGCCTACGAC-3′	409–429	202
		5′-CCAGCTTGTCCTTCCCGTTCT-3′	610–590	
HLA-DQB1	NM_002123.2	5′-GTCATCTCCTTTCATCCCCA-3′	979–998	121
		5′-AAACAGAAACCCCTTGGG-3′	1099–1082	
CCL19	NM_006274.2	5′-AGCAGTTAACCTATGACCGTGC-3′	427–448	245
		5′-CCAGGCGGCTTTATTGGTAGC-3′	671–651	
C3	NM_000064.2	5′-ACGAATGCCAAGACGAAGAG-3′	4966–4986	126
		5′-CTGAAGCTTTATCTGGAGTGGG-3′	5091–5070	
TNFSF10	NM_003810.2	5′-GATCAAGACCATAGTGACCAA-3′	1483–1503	167
		5′-TGGCATGATCTCACCACAC-3′	1649–1631	
18S	NR_003286.1	5′-GTAACCCGTTGAACCCCATTC-3′	1577–1597	153
		5′-CCATCCAATCGGTAGTAGCG-3′	1729–1710	

*Genebank accession number for the sequences used in designing the primers.

of immune regulatory genes between first- and second-trimester human fetal pancreas by microarray analysis.

RESEARCH DESIGN AND METHODS

Source of fetal tissue. For the transplantation experiments, human fetal pancreata were obtained from the therapeutic termination of pregnancies between 8 and 20 weeks gestation with informed maternal consent. The use of the tissue for research purposes was approved by the Human Research Ethics Committee of the Prince of Wales Hospital and University of New South Wales (UNSW).

For the microarray and PCR experiments, human fetal pancreata were obtained from the therapeutic termination of pregnancies between 10 and 23 weeks of gestation, according to protocols approved by the Health Sciences Research Ethics Board at the University of Western Ontario.

Experimental animals. Male NOD/SCID mice were obtained from the Animal Resources Centre (Perth, Australia) and maintained under sterile conditions. The experiments were approved by the animal ethics committee of UNSW.

Pancreas transplantation. NOD/SCID mice were anesthetized with 70 mg/kg pentobarbitone, and the pancreatic tissue was transplanted beneath the renal capsule as described previously (6).

Diabetic mice. First- and second-trimester human fetal pancreas was transplanted under the renal capsule of NOD/SCID mice. Two weeks later, diabetes was induced by the daily injection of 70 mg/kg body wt streptozotozin (Alexis Biochemicals, San Diego, CA) for 4 consecutive days. Mice with BGLs >16 mmol/l on two consecutive readings were accepted to be diabetic. The body weight and random BGLs of transplant recipients were measured three times per week. Mice were given 0.5 unit insulin subcutaneously if their random BGL readings were >16 mmol/l and were noted not to lose much weight despite being hyperglycemic. The mice were considered to be euglycemic when the BGL was <6 mmol/l on at least two consecutive measurements. After normalization, an oral glucose tolerance test was performed to confirm the euglycemic state. The test was carried out after a 4-h fast with the mice being given glucose (3 mg/g of 300 mg/ml glucose solution) orally. Blood samples were collected at 0, 20, 40, 60, and 120 min after glucose administration. To demonstrate that normalization was due to the graft, the kidney containing the graft was removed, and the BGLs were monitored for a further 2–3 days.

Humanization of mice. One week after NOD/SCID mice had been transplanted with the human fetal pancreatic tissue, the mice were constituted with human immune cells. Human PBMCs were obtained from whole blood by Lymphoprep Ficoll density gradient centrifugation (Axis-Shield PoC AS, Oslo, Norway). The PBMCs were resuspended in PBS at a concentration of 5×10^7 cells/ml and were immediately injected intraperitoneally into the transplanted NOD/SCID mice. All mice were humanized with PBMCs from the same male, nondiabetic donor who was tested negative for autoantibodies against islet cell antibody 512 and GAD islet cell antigens.

Fluorescence-activated cell-sorting analysis. Three weeks after PBMC injection, the spleen, bone marrow, peripheral blood, and one-half of the grafted pancreas were harvested, and single-cell suspensions were prepared by mechanical disruption. Erythrocytes were lysed using 10% fluorescence-activated cell-sorting (FACS) lysing solution (BD Biosciences, Sydney, Australia).

The following antibodies from BD Biosciences were used for the staining: anti-human CD45 allophycocyanin (APC)-conjugated (clone HI30), anti-mouse CD45 fluorescein isothiocyanate (FITC)-conjugated (clone 30-F11), anti-human CD19 phycoethrin (PE)-conjugated (clone HIB19), and the MultiTEST CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC kit. The cells were incubated with the respective antibody for 30 min and analyzed using a FACscaliburII sorter and Cell Quest FACS analysis system (BD Biosciences). Up to 50,000 events were analyzed per sample. As negative controls, the cell suspensions were stained with the respective isotype control for each antibody. The engraftment of human $CD45^+$ cells in the analyzed tissues was calculated as the percentage of the total number of $CD45^+$ cells (mouse plus human) detected.

Immunohistochemical staining. Three weeks after humanization, the grafted fetal pancreatic tissue was harvested, and one-half of the grafts were fixed in 10% formalin and embedded in paraffin for histology. The grafts were sectioned (6 μm) and stained with hematoxylin-eosin (H-E). Immunohistochemical staining was performed with a monoclonal antibody specific for human CD45 (N1514; Dako, Sydney, Australia) and a guinea pig anti-insulin antibody (A0564; Dako) using the avidin biotin-peroxidase system (LSAB-2 kit; Dako) according to the manufacturer's instructions. Negative controls were prepared omitting the primary antibody.

Immunofluorescent staining. Paraffin sections (5 μm) of human fetal pancreas at different developmental stages were immunostained using immunofluorescence protocols as described previously (19). Sections were stained with a mouse anti-human HLA-DP, DQ, DR antibody (Dako) and a secondary fluorescein (FITC)-labeled anti-mouse antibody (Jackson Immunoresearch Laboratories, West Grove, PA).

Affymetrix microarray analysis. For the microarray and PCR experiments, human fetal pancreas was divided into three developmental stages: first trimester (up to 13 weeks of gestation, $n = 5$), early second trimester (14–19 weeks, $n = 5$), and late second trimester (20–23 weeks, $n = 6$).

Total RNA was extracted using TRIZOL reagent (Invitrogen, Burlington, Canada). The quality of the RNA was verified by agarose gel electrophoresis and by Agilent 2100 Bioanalyzer Scans using the RNA 6000 Nano kit RNA quality (Caliper Life Sciences, Mountain View, CA). Biotinylated complimentary RNA (cRNA) was generated from 100 ng total RNA following cDNA in vitro transcription, using the BioArray High-Yield RNA Transcript Labeling kit (Enzo Biochem, New York) incorporating biotinylated UTP and CTP. The labeled cRNA was hybridized to Affymetrix HG-U133A GeneChips (Affymetrix, Santa Clara, CA), according to the manufacturer's instructions. Affymetrix GCOS 1.2 and GeneSpring v7.1 software were used to examine expression levels and perform statistical analyses. Data were then normalized using robust multichip analysis (RMA) to show relative expression to first-trimester tissue. Data normalization was used to standardize the data to allow any biological variation seen in gene expression to be independent from variation arising from the measurement process. For the replicates within a stage, a median value for the expression of each gene was taken to establish an average expression level. The RMA data were used for all further analysis, and data are expressed as mean fold changes (means ± SE).

RT-PCR and real-time RT-PCR. Real-time RT-PCR was performed, as described previously (20). For each RT reaction, 2 μg DNA-free RNA was

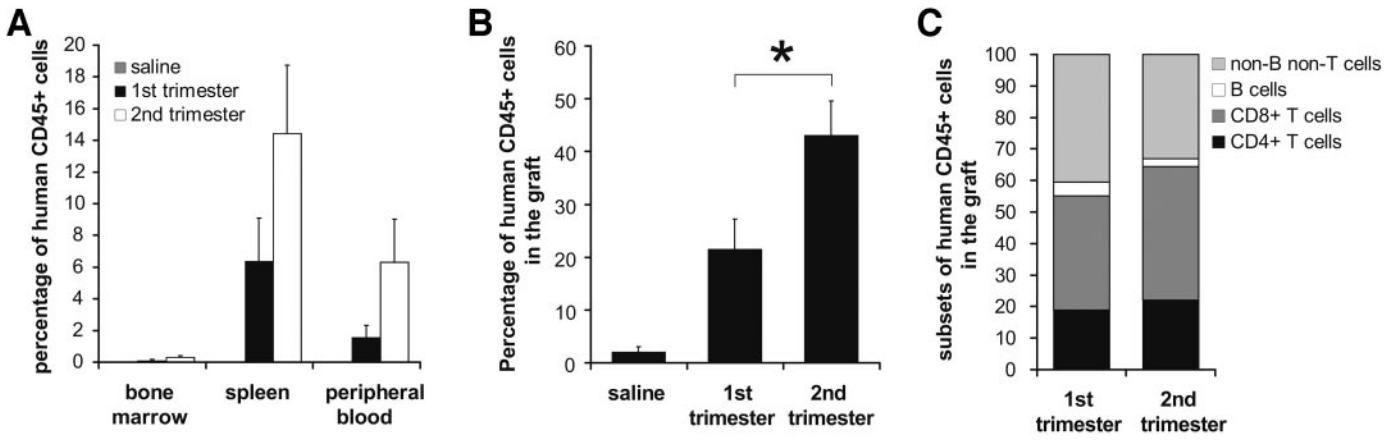

FIG. 1. Immunogenicity of first- and second-trimester human fetal pancreas. NOD/SCID mice were transplanted with first-trimester ($n = 7$) and second-trimester ($n = 7$) human fetal pancreas and 1 week thereafter reconstituted with 5×10^7 human PBMCs. Three weeks after humanization the graft and various tissues of the mice were analyzed for the presence of human mononuclear cells. *A*: FACS analysis for the presence of human CD45$^+$ cells in bone marrow, spleen, and peripheral blood of the mice. *B*: FACS analysis for the presence of human CD45$^+$ cells in the grafted human fetal pancreas. *$P < 0.05$. *C*: The subsets of human CD45$^+$ cells present in the grafts were determined by FACS analysis using specific antibodies against human CD19 (B-cells), CD8 (CD8$^+$ T-cells), and CD4 (CD4$^+$ T-cells).

used. The PCR primers used are listed in Table 1. Real-time RT-PCR analyses were performed with 0.1 μg cDNA using the iQ SYBR Green Supermix kit in Chromo4 Real Time PCR (Bio-Rad Laboratories, Mississauga, Canada). Data were normalized to levels of the 18S rRNA subunit. The relative gene expression was calculated based on the $2^{-\Delta\Delta C}{}_T$ method as relative PCR signals to first-trimester tissue (21). Controls involved omitting reverse transcriptase, cDNA, or DNA polymerase and showed no reaction bands.

Statistical analysis. Comparison between two groups was evaluated using Student's *t* test. For the real-time PCR data where more than two groups are compared, data were subjected to analysis of variance by one-way ANOVA followed by the post hoc least significant difference test. Results were considered statistically significant at *P* value of <0.05. All values are expressed as means ± SE.

RESULTS

FACS analysis shows a reduced immune reaction to first-trimester human fetal pancreas. To examine the immunogenicity of first-trimester human fetal pancreas, a humanized mouse model was established. We determined that the optimal number of human PBMCs to achieve engraftment in NOD/SCID mice was 5×10^7 and that second-trimester human fetal pancreas, which is known to be immunogenic, was rejected after 3 weeks when transplanted in this model (data not shown).

To compare the immune reaction of first- and second-trimester human fetal pancreas, the tissues were transplanted under the renal capsule of NOD/SCID mice, which were humanized by the injection of 5×10^7 human PBMCs 1 week thereafter. After 3 weeks, the engraftment of the human cells in the bone marrow, spleen, and peripheral blood of the mice and the engrafted pancreas was determined by FACS analysis. Mice transplanted with first-trimester pancreas consistently showed lower levels of CD45$^+$ cells engrafted in bone marrow (0.11 ± 0.05 vs. 0.29 ± 0.08%), spleen (6.37 ± 2.73 vs. 14.41 ± 4.33%), and peripheral blood (1.55 ± 0.78 vs. 5.25 ± 2.34%) compared with mice engrafted with second-trimester pancreas (Fig. 1*A*). However, the differences were not significant. Mice receiving saline injections instead of PBMCs demonstrated no engraftment of human cells in these tissues (Fig. 1*A*).

The number of human CD45$^+$ cells in the grafts of first-trimester human fetal pancreas, however, was significantly lower with 21.51 ± 5.73% compared with 43.11 ± 6.49% ($P < 0.05$) in the second-trimester grafts (Fig. 1*B*). Mice receiving a saline injection instead of human PBMCs demonstrated a very low level of human CD45$^+$ cells in the graft (2.01 ± 0.91%) (Fig. 1*B*). These cells represent the endogeneous CD45$^+$ cells present in the grafts at the time of transplantation, and the results demonstrate that the grafts themselves contain very few CD45$^+$ cells.

The subsets of CD45$^+$ cells in the grafts were analyzed by FACS staining for a B-cell (CD19), cytotoxic T-cell (CD8), and helper T-cell (CD4) marker. We found that the proportion of these cells present in the grafts was not significantly different between first- and second-trimester grafts. CD45$^+$ cells infiltrating the graft consisted mostly of CD8$^+$ T-cells (36.3 ± 4.3 and 42.4 ± 3.2%) and CD4$^+$ T-cells (18.7 ± 3.1 and 22.0 ± 1.9%) with only low numbers of B-cells (4.5 ± 1.4 and 2.4 ± 0.5%) (Fig. 1*C*). We also did not find any significant differences in the subsets of human CD45 cells in the spleen, bone marrow, and peripheral blood of the mice, and the proportions of the cell types were similar to what was found in the grafts (data not shown).

Histological analysis reveals low levels of infiltration in first-trimester human fetal pancreas transplanted in humanized mice. The histological examination of the grafts by H-E staining revealed extensive cellular infiltration in second-trimester grafts (Fig. 2*B* and *D*). Consistent with the FACS analysis data, an extensive human CD45$^+$ infiltrate could be observed (Fig. 2*J* and *L*). As expected, the second-trimester grafts were completely rejected by 3 weeks with no pancreatic ducts or insulin-positive cells detectable (Fig. 2*D*, *F*, and *H*).

In contrast in the first-trimester grafts, only minimal infiltration occurred (Fig. 2*A* and *C*). The infiltrating human CD45$^+$ cells were almost exclusively restricted to the graft-kidney interface with few CD45$^+$ cells within the grafts (Fig. 2*I* and *K*). The integrity of the first-trimester grafts was still intact with abundant ductal epithelial structures (Fig. 2*C*), consistent with healthy human fetal pancreas. Additionally, the first-trimester grafts contained numerous insulin-producing cells scattered throughout the grafts (Fig. 2*E*). The insulin-positive cells were present as single cells within ducts as well as small clusters of cells (Fig. 2*G*), which is similar to the pattern of insulin staining of human fetal pancreas at this developmental stage (22,23). These results clearly show that first-trimester

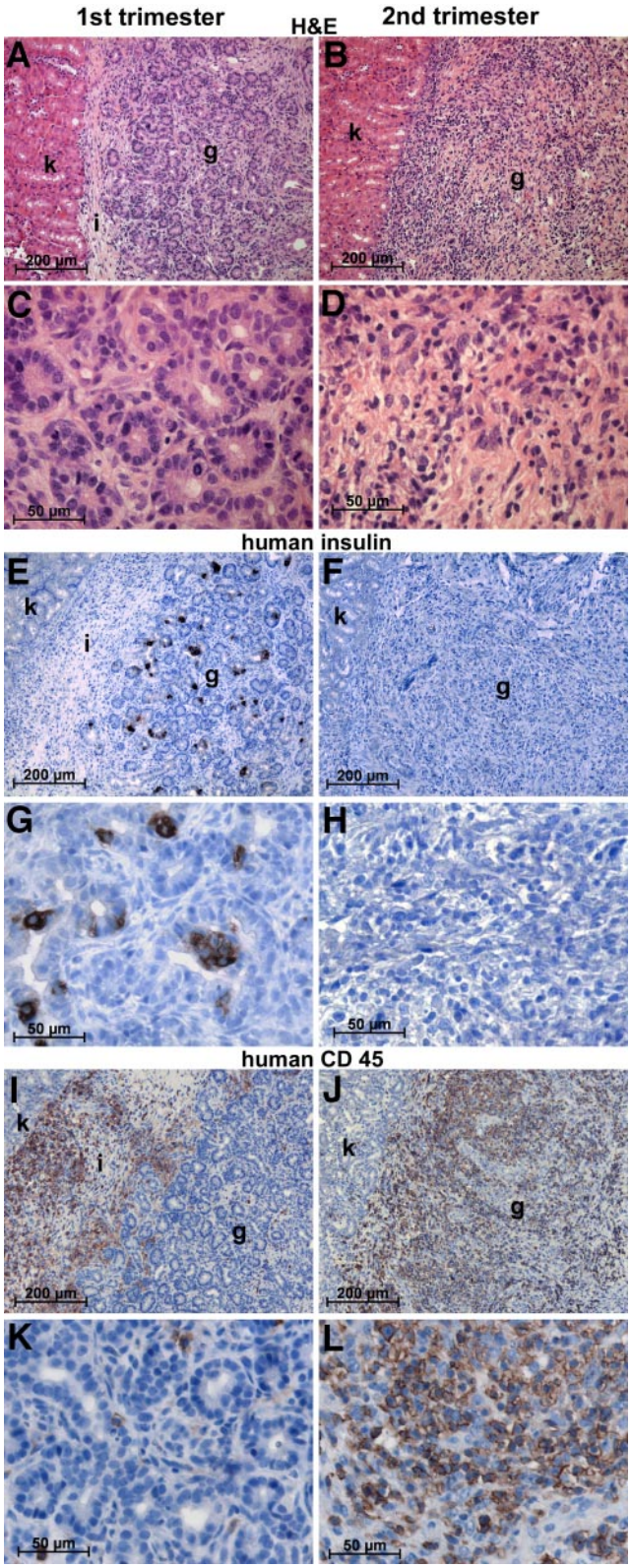

FIG. 2. Histological analysis of first- and second-trimester human fetal pancreas grafts. Transplanted human fetal pancreata were recovered 3 weeks after injection of 5×10^7 human PBMCs. Representative pictures of an 8-week-old first-trimester and a 16-week-old second-trimester graft are shown. *A–D*: H-E staining of the grafts at low (*A* and *B*) and high (*C* and *D*) magnification. *E–H*: Insulin staining (brown) of the grafts at low (*E* and *F*) and high (*G* and *H*) magnification. Note the absence of insulin in the second-trimester graft. *I–L*: Grafts were stained with an anti-human CD45 antibody (brown) to assess the level of infiltration. g, human fetal pancreas graft; i, kidney-graft interface; k, murine kidney. (Please see http://dx.doi.org/10.2337/db07-0720a for a high-quality digital representation of this figure.)

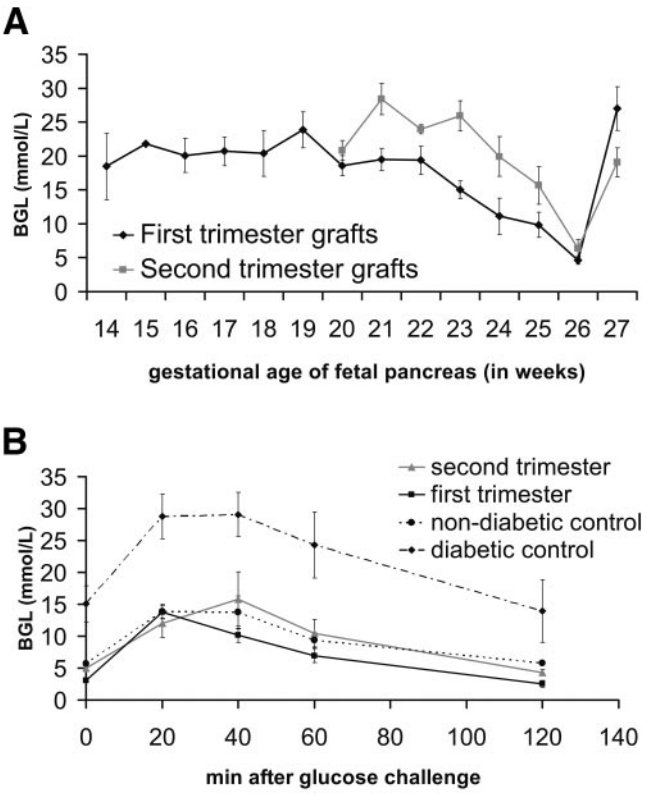

FIG. 3. Comparison of the functional development of first- and second-trimester human fetal pancreas. NOD/SCID mice were transplanted with first- ($n = 4$) and second-trimester ($n = 4$) human fetal pancreas and 2 weeks thereafter made diabetic. *A*: The average weekly BGLs are shown. On normalization (BGL <6 mmol/l), a nephrectomy was performed. BGLs are shown only after induction of diabetes. *B*: An oral glucose tolerance test was performed after normalization of random BGL. Nongrafted, nondiabetic, and diabetic NOD/SCID mice were taken as controls.

human fetal pancreas is not rejected when transplanted in humanized mice during a period of 3 weeks, whereas second-trimester human fetal pancreas is completely rejected within this period.

Normalization of BGLs by first-trimester human fetal pancreas. To determine the capacity of first-trimester human fetal pancreas to control hyperglycemia in comparison with second-trimester fetal tissue, NOD/SCID mice were transplanted with first- and second-trimester pancreata. After 2 weeks, diabetes was induced in the animals by injection of streptozotozin, which was shown to have no adverse effect on human fetal pancreas (24). In the mice transplanted with second-trimester pancreas, the BGLs normalized within 6–8 weeks (Fig. 3*A*). The mice transplanted with first-trimester pancreas took 8–14 weeks to reverse diabetes (Fig. 3*A*). However, despite the age of the initially transplanted graft, all of the grafts were able to reverse diabetes at a gestational age of around 26 weeks. Oral glucose tolerance tests confirmed the euglycemic state, with no differences between the first trimester, second trimester, and nondiabetic control groups (Fig. 3*B*).

Differential expression of immunoregulatory genes in first- and second-trimester human fetal pancreas. To investigate whether a decreased expression of immunoregulatory genes in the first-trimester tissue might account for its reduced immunogenicity, a microarray analysis of gene expression in human fetal pancreas at different developmental stages was performed. First-trimester tissue of the gestational age up to 13 weeks was

TABLE 2
Expression of selective immune-related genes with an >1.5-fold difference in the developing human fetal pancreas

Gene title and category	Early second trimester	Late second trimester
MHC class II molecules		
HLA-DQB1	1.53	1.57
HLA-DRB1	2.15	1.6
HLA-DRB4	1.74	1.47
HLA-DRB5	1.79	1.49
Chemokines and adhesion molecules		
Chemokine ligand 19 (CCL19)	2.07	1.54
N-Cadherin (CDH2)	−2.5	−2.9
Neuronal cell adhesion molecule (NRCAM)	1.65	1.87
Trophinin (TRO)	−2.7	−3.7
Innate molecules		
Immune costimulatory protein B7-H4	3.04	3.81
Immunoglobulin κ constant (IGKC)	1.72	2.03
Immunoglobulin superfamily member 1 (IGSF1)	1.76	1.89
Immunoglobulin superfamily member 3 (IGSF3)	−1.67	−2.17
Complement component		
Complement component 3 (C3)	2.07	1.60
Mannose receptor C type (MRC1)	−1.64	−2.44
ILs		
IL 22 receptor α1 (IL22RA1)	1.96	3.18
IL enhancer binding factor 2 (ILF2)	−1.96	−2.44
Apoptotic markers		
TNFSF10	1.91	1.74

Data are fold change and are normalized to first trimester.

compared with early second-trimester tissue (14–19 weeks of gestation) and late second-trimester tissue (20–23 weeks of gestation). We identified 104 genes with immunoregulatory function that had at least a 1.5-fold difference in expression in second-trimester pancreas compared with first-trimester tissue. Of these genes, 53 were downregulated and 51 upregulated in the older tissue. A list of the genes is provided in the online appendix, which is available at http://dx.doi.org/10.2337/db07-0720a.

Table 2 gives a representative overview of 17 immunoregulatory genes of interest that were found to be differentially expressed in the analyzed pancreatic tissues (Table 2). The expression of six of these genes was validated by quantitative real-time RT-PCR.

Major histocompatibility complex (MHC) class I and II molecules are among the most potent antigens in allograft rejection (25). Four MHC class II molecules were found to be upregulated between 1.5- and 2-fold in early and later second-trimester human fetal pancreas (Table 2). The expression of the MHC class II molecule HLADQB1 was confirmed to be significantly unregulated by ~fivefold (early second trimester) and ~fourfold (late second trimester) by real-time RT-PCR (Fig. 4*B*). This increased expression of HLA class II molecules in early and late second-trimester human fetal pancreas was further verified by immunofluorescent staining, which showed an increasing number of cells expressing HLA class II molecules scattered throughout the second-trimester pancreas (Fig. 5). This could be an indication of a greater number of passenger leukocytes in the second-trimester grafts.

In contrast to MHC class II, MHC class I molecules were not significantly upregulated in the microarray analysis, although there was a trend for this to occur (see online appendix). Quantitative RT-PCR confirmed this for HLA-B and for early second-trimester HLA-Abut showed that the trend reached significance for late gestational second-trimester pancreas (Fig. 4*E* and *F*).

We also analyzed the expression of chemokine ligand 19 (CCL19) and complement component 3, because they are involved in recruiting T-cells and regulating their response (26,27), and the tumor necrosis factor (TNF) superfamily member 10 (TNFSF10 or TNF-related apoptosis-inducing ligand [TRAIL]), which has been reported to have costimulatory activity on T-cells (28,29). The upregulation of all of these genes in the second-trimester human fetal pancreas as seen in the microarray was confirmed by real-time RT-PCR. All of these molecules were expressed at significantly higher levels in both early and late second-trimester human fetal pancreas (Fig. 4*A*, *C*, and *D*) with no significant differences between the early and late second-trimester groups.

DISCUSSION

Fetal tissue has been widely discussed in recent years as an alternative source of tissue for transplantation due to the severe shortage of adult donor tissue available. In this study, we analyzed the immunogenicity of first-trimester human fetal pancreas as a potentially new treatment for type 1 diabetes. We used the human PBMC reconstituted mouse model, an established model to study the allogeneic rejection of tissue (11,13,30). We demonstrated that human fetal pancreas obtained from the first trimester of gestation (up to 13 weeks) is significantly less immunogenic than second-trimester tissue (14–26 weeks of gestation). When transplanted into humanized mice, second-trimester pancreas is completely rejected within 3 weeks, characterized by extensive infiltration of human mononuclear cells of the graft. The infiltrating cells consisted mostly of cytotoxic T-cells and helper T-cells. This has also been the observation in a study that used a different humanized mouse model to determine the rejection of second-trimester human fetal pancreas. The tissue was rejected within 4 weeks after being transplanted, and the rejection was mediated by cytotoxic and helper T-cells (13). Our study, however, is the first report on the immunogenicity of human fetal pancreas obtained during the first trimester, and our results show that compared with second-trimester tissue, first-trimester human fetal pancreas is significantly less immunogenic. Infiltration of human mononuclear cells in the graft was only minimal and often restricted to the interface of the graft and the murine kidney.

It is generally believed that fetal tissues are less immunogenic than their adult counterparts, and previous studies have shown that minimal immunogenicity is observed by fetal tissues harvested at the earliest gestational time points possible (8,14,16). Dekel et al. (15) demonstrated in a humanized mouse model that adult and second-trimester renal grafts were completely rejected whereas early kidney precursors as young as 7 weeks of gestation showed no signs of rejection. Similar results have been shown in xenograft transplantations of fetal pig pancreas into humanized mice. Pancreatic tissue from embryonic day 42, which corresponds to early second-trimester tissue, showed markedly reduced immunogenicity compared

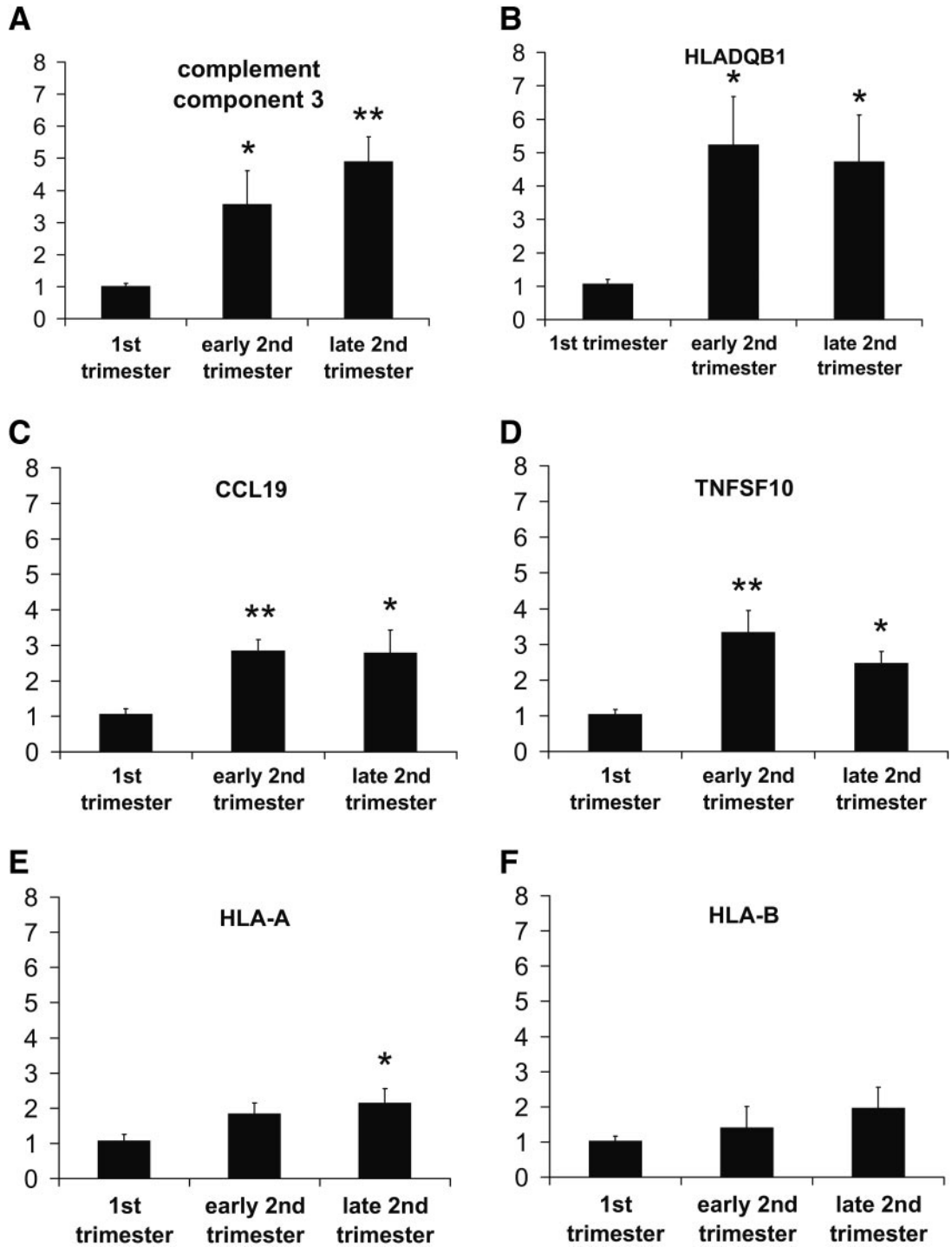

FIG. 4. Different expression of immunoregulatory genes in first- and second-trimester human fetal pancreas. The expression of immunoregulatory genes in first-trimester (up to 13 weeks), early second-trimester (14–19 weeks), and late second-trimester (20–23 weeks) human fetal pancreas was determined by quantitative real-time PCR analysis. The expression is given as relative expression compared with the first-trimester tissue. *A*: Complement component 3 (n = 8). *B*: MHC class II molecule HLADQB1 (n = 8–9). *C*: Chemokine ligand 19 (CCL19) (n = 7–8). *D*: TNF superfamily member 10 (TNFSF10) (n = 7). *E*: MHC class I molecule HLA-A (n = 5). *F*: MHC class I molecule HLA-B (n = 4–5); *P < 0.05 compared with first-trimester tissue; **P < 0.01 compared with first-trimester tissue.

with older tissue, was able to normalize BGLs in diabetic mice, and exhibited superior insulin secretion and growth after transplantation compared with younger tissue (16, 31). This demonstrates that tissues from specific windows of gestational development are more suitable for transplantation than others.

We have shown in this study that there are no functional differences between first- and second-trimester human fetal pancreas. Both have the same ability to normalize BGLs when transplanted into diabetic mice. For both first- and second-trimester human fetal pancreata, normalization occurred when the tissue had a gestational age of around 26 weeks, which is consistent with previous findings from our group (4). Castaing et al. (2) have also shown that human fetal pancreas at an age as early as 6–9 weeks of gestation has a remarkable capacity to grow and

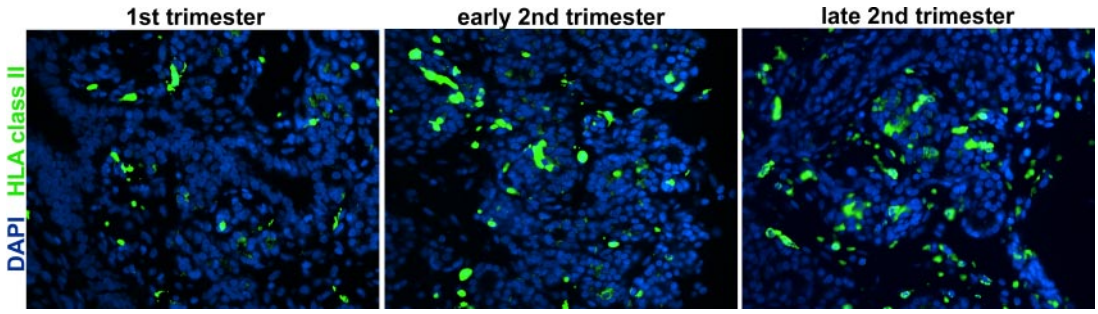

FIG. 5. Expression of HLA class II molecules in first- and second-trimester human fetal pancreas. Immunofluorescent staining of first-, early second–, and late second–trimester human fetal pancreas with an antibody against HLA-DQ, DR, and DP (green staining). Nuclei were stained with DAPI. Representative pictures of a first-trimester pancreas at 12 weeks of gestation (*A*), early second–trimester pancreas at 16 weeks of gestation (*B*), and a late second–trimester pancreas at 22 weeks of gestation (*C*) are shown. All pictures were taken at magnification ×400. (Please see http://dx.doi.org/10.2337/db07-0720a for a high-quality digital representation of this figure.)

is able to normalize BGLs of diabetic immunodeficient mice. Thus the reduced immunogenicity of the younger tissue makes it a potentially preferred candidate for transplantation.

However, although first-trimester human fetal pancreata were found to be less immunogenic than second-trimester tissue and reduced rejection of the grafts was observed, an immune response still did occur. It needs to be investigated whether the first-trimester grafts will be rejected as the grafts mature in vivo or whether tolerance will develop and the grafts will be accommodated by their hosts.

If pig pancreatic primordia are obtained before embryonic day 35, which corresponds to first trimester, they engraft in immunocompetent diabetic rats without the need for immunosuppression (32). In a different study the administration of immunosuppressive drugs affecting costimulatory blockade resulted in the long term survival of early gestational pig pancreatic grafts in humanized mice but not in the survival of older tissue (16). Although the mechanisms of xenograft rejection are different from allograft rejection, these results give hope that the need for immunosuppression might be reduced with the use of first-trimester human fetal pancreas. The possibility that tissue obtained during early stages of development might be tolerated by the host when transplanted is supported by the results from the human fetal kidney study, where allograft rejection of early kidney precursors in humanized mice was not seen even after a second injection of PBMC from a different donor (15). Molecular biological studies of the early fetal kidneys revealed an immaturity of the tissue in terms of immunoregulatory gene expression compared with adult renal tissue with several immune-related genes expressed significantly lower in the fetal kidneys (15). In the present study, we also found a significant downregulation of several immune-related genes in the first-trimester fetal pancreas compared with second trimester, some identical to the genes demonstrated to be downregulated in the fetal kidneys, including MHC molecules, chemokines, complement components, and also TRAIL. These molecules are involved in recruiting T-cells and regulating their response (26,27), and TRAIL has been reported to have costimulatory activity on T-cells (28,29). The MHC class II molecules in particular are involved in triggering an immune response. They were upregulated in the second-trimester human fetal pancreas, perhaps because of a greater number of passenger leukocytes present in the second-trimester fetal pancreas. This and the altered expression of other genes that play a role in immune recognition and responses may account for the reduced immunogenicity of first-trimester human fetal pancreas.

Taken together, our study is the first to demonstrate that first-trimester fetal pancreas displays reduced immunogenicity while having the same functional capacity compared with second-trimester tissue. These properties and the excellent proliferative capacity of first-trimester human fetal pancreas show its potential as a source for transplantable β-cells.

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