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FREQUENCY OF KIDNEY REJECTION IN DIABETIC PATIENTS UNDERGOING SIMULTANEOUS KIDNEY AND PANCREATIC ISLET CELL TRANSPLANTATION[1,2]

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An increased frequency of kidney rejection has been reported in diabetic patients who have simultaneous pancreas and kidney transplantation compared with patients who have a kidney transplant alone. Kidney graft outcome is similar in the two groups. The mechanism for increased kidney graft rejection with a simultaneous pancreas graft is not clear. It is ascribed to the immunogenicity of the exocrine pancreas that initiates migration of activated cells from the peripheral blood that are entrapped in the kidney. Since the volume of the transplanted tissue is less in islet transplantation (usually <2 ml) than in pancreas transplantation, one might not expect an increased frequency of kidney rejection in islet cell recipients. We looked at biopsy-proven kidney rejection episodes in patients who had combined kidney and islet transplants and compared this with the frequency of rejection in diabetic and nondiabetic patients who underwent a kidney transplant alone under the same immunosuppression. Diabetic patients who had kidney islet transplants (n=9) had a higher frequency of rejection (100%) compared with diabetic patients (n=107, 55.1%) and nondiabetic patients (n=327, 65%) who had a kidney transplant alone. The 1-year graft and patient survival rates were not different among the groups. Although the number of patients is small, it would appear that transplantation of a low volume of islet cells with high purity can lead to an increased frequency of kidney rejection. This is unlikely to be explained solely on the basis of fewer antigen matches in these recipients but may reflect the inherent immunogenicity of the purified islet preparations. Alternatively, there may be an effect of their direct infusion into the portal vein.

When a normal kidney is transplanted into a diabetic patient with abnormal glucose metabolism, characteristic changes induced by diabetes occur in the transplanted kidney over a variable period, sometimes faster than the time of onset noted in native kidneys (*1*). This consequence can be avoided if a

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successful pancreas transplant is performed restoring euglycemia (*2*, *3*). It has been reported that there is no overall adverse effect of performing pancreas transplant on the outcome of the patient or the transplant kidney (*4*, *5*). With the increasing success of pancreas transplantation, this approach is increasingly accepted as the closest approximation of the ideal of long-term restoration of normal metabolism. While the long-term kidney graft outcome is similar in diabetic patients undergoing combined pancreas and kidney grafts, there are many series that show an increased frequency of acute kidney rejection episodes in this group of patients (*6–8*). This has not been noted in all series (*9*, *10*). The mechanism of how the pancreas graft might induce rejection in the transplanted kidney is not known; however, on the basis of experimental evidence it has been proposed that activated cells from the circulation migrate and lodge in the transplanted kidney (*11*).

While this is controversial, exocrine tissue probably contributes significantly to the immunogenicity of islet preparations (*12–16*). Since the volume of nonislet cells is much lower with an islet graft than with a whole pancreas graft, an increased frequency of kidney rejection might not be expected in patients undergoing islet transplantation. It was therefore of interest to examine the frequency of kidney rejection episodes in patients who underwent combined kidney and purified islet transplantation.

MATERIALS AND METHODS

Patient characteristics. Eight patients aged 29–38 years with long-standing insulin-dependent (type I) diabetes mellitus as evidenced by an absent C-peptide response to either glucagon or Sustacal stimulation received 9 combined cadaveric kidney-islet grafts (one retransplant), with one (n=6), two (n=2), or three (n=1) islet donors. The cadaveric donor ABO types were all compatible with recipient types and HLA matching was random, the antigen match being 0–2 for the kidney and 0–3 for islets (Table 1). All patients had a negative crossmatch. One patient who underwent the procedure died on the fifth postoperative day of aspiration pneumonia and did not have rejection until this time. This patient was not included in the analysis of frequency of rejection, but was included in the calculation of mortality and graft survival.

Islet isolation and transplantation. Human islets were obtained by a modification (*17*) of the automated method for human islet isolation (*18*) and purified by centrifugation on discontinuous density gradients (Eurocollins-Ficoll, density=1.108, 1.096, 1.037) using a Cobe 2991 cell separator (Cobe, Lakewood, CO) (*19*, *20*). Immediately after renal transplantation, an upper midline incision was performed and a 16-gauge catheter was placed in a jejunal vein for islet infusion. In one patient (No. 3) the catheter was left in place for transplantation of islets from a third-party donor.

Patient 5 received islets via the transhepatic route from a third-party donor, since islets of sufficient quality could not be obtained from the pancreas of the kidney donor. The transhepatic route was also used to transplant islets from a second donor in patient 8. Islets were all transplanted fresh or after overnight culture at room temperature.

Immunosuppression. All patients received a 100-mg bolus of methylprednisolone during the operation, followed by a decreasing prednisone dose from 200 mg to 20 mg over the course of the first week posttransplant. FK506 was given at a dose of 0.1 mg/kg intravenously administered as a continuous infusion over 24 hr, beginning immediately following the transplantation. When patients resumed a solid diet, an oral dose of 0.15 mg/kg/bid was started. Patients 6–8 also received Imuran 200 mg/day during the first postoperative week in addition to the previously mentioned immunosuppression.

Definition of rejection. Kidney biopsy was performed to confirm a clinical impression of rejection, and the tissue was processed immediately in formalin and stained with hematoxylin and eosin, Jones, trichrome, and PAS stains. The only rejection episodes considered in this report were those confirmed histologically by biopsy. Rejection episodes were graded as mild, moderate, or severe as follows. Mild acute cellular rejection was considered as: small aggregates of mononuclear cells with evidence of tubulitis. Moderate: same as above with some coalescence of mononuclear aggregates. Severe: same as above with evidence of necrosis, interstitial hemorrhage, and acute inflammation or vasculitis.

Statistics. Data were analyzed by chi square test with continuity correction. Significance of difference was considered as $P < 0.05$.

TABLE 1. Characteristics of diabetic patients who underwent combined kidney-islet transplantation

Patient No.	Age	Sex	Duration of diabetes mellitus	Procedure[a]	HLA matches
1	38	M	22	KI	A2
2	29	M	23	KI+I	Donor 1 (KI): DR1:DR4
					Donor 2 (I): DR4
				KI	Donor 3 (KI): B7
3	34	M	26	KI+I, I	Donor 1 (KI): A2
					Donor 2 (I): A2, B8, DR3
					Donor 3 (I): A2, B8, DR3
4	36	F	15	KI	None
5	36	M	17	K,I	Donor 1 (K): none
					Donor 2 (I): DR3
6	32	M	18	KI	None
7	32	M	22	KI	None
8	29	M	16	KI,I	Donor 1 (KI): A2, B62
					Donor 2 (I): A2, DR4

[a] KI: simultaneous kidney + islets. KI+I: simultaneous kidney + islets + islets from another donor. K,I: kidney with islets from a separate donor.

RESULTS

Six-month graft survival was 86%, 76%, and 78% in diabetic recipients of a solitary kidney graft (DK),* nondiabetic kidney transplant recipients (NDK), and diabetic recipients of kidney and islets (DKI), respectively (Table 2). One-year graft survival was 82% (DK), 73% (NDK), and 78% (DKI). Mortality rates were not different in the groups. The unexpected finding was the frequency of kidney rejection episodes: 55.1% in DK, 65% in NDK, and 100% in DKI patients ($P < 0.02$).

The number and grade of biopsy-proven rejection episodes and current graft outcome in DKI patients are shown in Table 3. Most episodes were mild and easily treated with corticosteroids. Only one graft was lost to refractory recurrent rejection episodes. After rejection episodes, 5 patients still have demonstrable islet cell function, but all patients still require exogenous insulin. The best results were obtained in the patients who received islets from more than one donor pancreas.

In order to examine the rejection frequency in more detail, a matched case control study from the original group was done. Diabetic (n=9) and nondiabetic (n=9) controls were randomly selected for each kidney-islet recipient using matching criteria of age, sex, and time of transplant. The frequency of kidney rejection episodes was compared between kidney islet patients and controls using McNemar's test. Mean and median creatinine, FK dose, and FK levels were compared between the groups using a one-way repeated measure analysis of variance and Friedman's nonparametric test (*21*).

* Abbreviations: DK, diabetic kidney recipient; DKI, diabetic kidney and islet recipient; NDK, nondiabetic kidney recipient.

TABLE 2. Comparison of graft and patient survival and frequency of rejection episodes

	Diabetic (n=107)	Nondiabetic (n=327)	Diabetic kidney and islet (9 kidney grafts, 8 patients)
Graft survival (%)			
6 months	86%	76%	78%
1 year	82%	73%	78%
1-year mortality rate	10.3%	6.7%	12.5%
Frequency of kidney rejection	55.1%	65%	100%*

* $P<0.02$ (chi square).

TABLE 3. Outcome in diabetic patients who underwent kidney-islet transplantation

Patient no.	No. rejections	Time (days)	Severity	Kidney outcome	Islet outcome
1	2	34, 48	Mild	Creat. 2.0	(+) C-peptide
2	3	11, 65, 75	Mild, mod	Graft failed	(+) C-peptide
	3 (2nd Tx)	8, 14, 425	Mild, mod	Creat. 1.6	(+) C-peptide
3	1	12	Mild	Creat. 1.9	(+) C-peptide
4	2	20, 240	Mild, mod	Creat. 1.3	(−) C-peptide
5	4	10, 40, 63, 94	Mild, mod	Creat. 2.2	(−) C-peptide
6	2	10, 72	Mild	Creat. 2.0	(+) C-peptide
7	1	18	Mild	Creat. 2.0	(−) C-peptide
8	1	105	Mild	Creat. 1.8	(+) C-peptide

Again, the frequency of rejection was higher in DKI recipients—100% vs. 55.6% in DK case controls and 33.3% in NDK case controls. The FK dose was 13.3±5.9 mg/day in DKI, 10.7±7.1 mg/day in DK, and 14.0±9.1 mg/day in NDK ($P>0.05$). The FK levels were also similar in the groups: 0.6±0.3 ng/ml (DKI), 0.6±0.6 (DK), and 0.8±0.4 (NDK) ($P>0.05$). Creatinine levels at a similar time point were no different among the groups.

DISCUSSION

Although the number of patients in our kidney islet group is small, the frequency of rejection episodes seems out of proportion to what was expected for islet transplantation. As with the rejection episodes seen in patients with kidney and pancreas grafts, these rejection episodes are largely manageable, and graft and patient outcomes are similar in the groups studied.

With this small number of patients it was not possible to analyze if significant differences in HLA matches were present in the three groups. In the recipients of kidney transplant alone, 2/3 of the patients had 2-antigen matches or less, similar to our patients.

It has been assumed in whole pancreas transplant that the exocrine gland initiates the recipient response and that reactive cells migrate to the kidney and possibly provide some protection to the pancreas (*11*). The reactivity to islets was supposed to be minimal.

In light of these data showing an apparent increased frequency of kidney rejection, the immunogenicity of the islet preparation and the intravascular route of administration (portal vein) must be considered as possible determining factors. Despite the small volume and relative apparent purity of the islet preparations, significant numbers of contaminating cells are still present. The potentially immunogenic cells include acini, ducts, lymphoid, and dendritic cells (*22*).

The intravascular administration of antigen and cells may facilitate antigen recognition and activate the immune response by direct presentation of antigen to circulating recipient lymphoid cells. The site of the intravascular antigen and cell administration may also be a key since in other systems, the portal venous route produces different immune responses than systemic venous administration (*23*).

Our data also indicate that a single mild rejection episode, even in a patient who receives islets from multiple donors, may be enough to compromise the ability to achieve complete insulin and independence, although diabetes control may be stabilized (*17*). It is therefore critical to develop effective procedures to decrease the immunogenicity of islet preparations before transplantation or to explore alternative sites or routes of administration. It will be of interest to determine if increased frequency of kidney rejection has been observed following transplantation of kidney and islets that have been cryopreserved or cultured for 1 week at 24°C. Experimental evidence suggests that these procedures may be effective pretransplant treatments to decrease islet preparation immunogenicity (*24*, *25*).

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DISCUSSION

DR. BRAYMAN (Philadelphia, Pennsylvania): Was the degree of HLA matching in the kidney transplant alone group, diabetic and nondiabetic, similar to the islet group?

DR. CARROLL: It is not possible to do statistics on the small number of patients that we had in the islet group, but two-thirds of the patients in the other two groups had zero to two antigen matches. That means that some patients had better matches, but most were similar to the patients who had kidney islet grafts.

DR. BRAYMAN: Could you comment about the severity of the rejection episodes in the kidney transplant alone versus the kidney islet group, do you have any idea whether they were similar or different?

DR. CARROLL: I think our patient numbers are still too small for conclusions. The whole spectrum is seen in the other groups also.

DR. BRAYMAN: Finally, since most rejection episodes occurred within the first three weeks after transplantation, would you comment on whether or not you think that an induction therapy with ALG or one of the monoclonals may be worthwhile?

DR. CARROLL: We have elected not to do that based on the transplant team's previous experience. Also, other groups involved in islet cell transplantation have been using induction therapy; the major problem that all of the groups currently face is rejection of the islet graft. This doesn't seem to be controlled by induction therapy. We're really focusing on efforts to alter immunogenicity of the islets rather than treating the patients with pharmacologic agents.

DR. RICORDI (Pittsburgh, Pennsylvania): Maybe we can learn something from the pancreas transplant experience in which the induction therapy with ALG has been used. An increased frequency of kidney rejection episodes were observed in most of the series. It is interesting to note that the only two series that did not report an increased frequency of kidney rejection after pancreas transplantation were those using duct occlusion techniques.

DR. BOUDREAUX (New Orleans, Louisiana): Did you find any depression of C-peptide levels or any other indication that something was happening to your islet grafts at the times of these kidney graft rejections?

DR. CARROLL: None of our patients have had rejection of islet independent of kidney rejection. The C-peptide drops when kidney rejection is occurring. And we have seen that with recycling of steroids, you can observe some recovery of function. But we have never seen a patient achieve insulin independence, even with one mild rejection episode.

DR. BOUDREAUX: Did you find a "stair-stepping" decline in islet function?

DR. CARROLL: Yes.

DR. GORES (Minneapolis, Minnesota): At the University of Minnesota between 1978 and 1991, we performed a dozen simultaneous islet/kidney transplants using our standard immunosuppression for cadaver kidney recipients, which included ALG; we didn't have any success with the islet grafts as far as achieving insulin independence. There was a 55 percent experience with acute renal rejection episodes, and that was not different than diabetic recipients of kidney transplants alone from cadaveric donors.

DR. CARROLL: It might be patient selection. I don't really know. The other variables would be, did you use one donor?

DR. GORES: We used one donor, and likely there was more exocrine tissue than in your series, but we used ALG induction, not just FK506 and prednisone. Perhaps that at least partially explains the difference.

DR. CARROLL: But the pancreas transplant people who use the same induction see the increased frequency of kidney rejection. You have reported it and other groups have reported it.

DR. GORES: The pancreas, but I'm talking about islets.

DR. CARROLL: Right.

DR. GORES: Using the same immunosuppression that Dr. Sullivan uses for the pancreas.

DR. CARROLL: It's interesting. Thank you.

DR. SUTHERLAND (Minneapolis, Minnesota): Your experience with renal rejection episodes in islet/kidney transplant recipients is similar to the experience with the pancreas/kidney transplants. You added islets without increasing intensity of induction immunosuppression with ALG or OKT3, and an extremely high incidence of rejection resulted. Indeed, the incidence of 100% is higher than with simultaneous pancreas/kidney transplants. I wonder if the purity of your islet preparations is less than you think. There may be sufficient contamination with lymphatic tissue so that the passenger leukocyte load to which the recipient is exposed is significantly increased over that associated with a kidney transplant alone.

One statement you made, that exocrine tissue is more immunogenic than islets, is not supported by experimental evidence. In some experimental transplant models, "dirty" islets have been associated with a higher frequency or more rapid rejection than pure islets. However, in most of these experiments, the "dirty" islets have included lymphatic and vascular tissue. Dr. Gores, who just commented, reported an experiment several years ago in which he deliberately added back exocrine tissue to the islet preparation, without the lymphatic or vascular or ductal tissue. When such tissue was transplanted, there was no increase in frequency or intensity of rejection over that observed when pure islets were transplanted, indicating that exocrine tissue itself did not increase immunogenicity. Dr. Monaco's laboratory has reported an increase in immunogenicity when impure islets are transplanted, but again the islet tissue was contaminated by lymphatic, vascular, and ductal tissue as well as exocrine tissue. When a whole pancreas is transplanted, the maximal load of exocrine tissue possible is transplanted, but the graft is also very contaminated with lymphatic tissue. In fact, a pancreas graft can actually induce graft versus host disease to some degree, particularly when a graft from a blood group O donor is transplanted to a non-O recipient, with induction of Coombs' positive hemolytic anemia. This has also been seen with other extrarenal organ transplants such as a liver, again an organ that harbors a large amount of lymphatic tissue. I think that a fair amount of lymphatic tissue also contaminates your islet preparation, and that is why incidence of rejection episodes is similar to what is seen with pancreas/kidney transplants.

DR. CARROLL: We have recently reported with Dr. Demetris and Dr. Sever, that the preparations contain ductal elements, dendritic cells, lymphoid tissue and much more than you would suspect. But it's still amazing that you can inject 800 microliters of a fairly pure preparation and get the same outcome as having a vascularized graft.

DR. SUTHERLAND: I agree.

DR. MONACO (Boston, Massachusetts): Well, I can't speak for the human, but in the mouse the evidence is overwhelming that exocrine tissue subtracted from a crude islet preparation is much more immunogenic than the purified islet preparation alone. If you hand-pick the islets, they survive longer than if you don't, furthermore, hand-picked islets are placed under one kidney capsule, and if you place the material remaining in the Petri dish under the other kidney capsule, the islets reject.

DR. CARROLL: I agree with you.