Allosensitization of Islet Allograft Recipients

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Background. The immune monitoring of islet transplant recipients includes the assessment of panel reactive antibodies (PRA). A negative association of PRA+ with allogeneic solid organ graft survival has been recognized, but scattered data is available for islet transplantation.

Methods. We performed a retrospective analysis of PRA status in 66 patients with type 1 diabetes mellitus recipient of islet allografts between 1985 and 2006.

Results. Pretransplant PRA+ was observed in 10 subjects in the old trials and associated with kidney transplantation and/or pregnancies. Thirteen subjects displayed PRA+ at follow-up, eight of whom were de novo. Overall, PRA+ did not correlate with islet graft outcome: long-term graft survival was observed in the presence of basal or persistent PRA+ and graft dysfunction occurred also in the absence of PRA+. Loss of graft function was associated with PRA+ after lowering of immunosuppression or after infection episodes. Loss of C-peptide did not affect kidney graft function even in simultaneous islet-kidney transplant recipients. Mostly, PRA remained negative under adequate immunosuppression. Patients whose immunosuppression was discontinued invariably developed PRA+.

Conclusions. Monitoring of PRA under immunosuppression may have little clinical value under adequate immunosuppression in islet transplant recipients. The implications of allosensitization after discontinuation of immunosuppression need to be evaluated to define the real clinical impact in this patient population.

Keywords: Islets of Langerhans, Islet transplantation, Allosensitization, Alloantibody, Panel reactive antibody, Human leukocyte antigens, Graft function, Diabetes, Type 1 diabetes.

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The past three decades have witnessed substantial progress in clinical islet transplantation (*1–4*) for patients with type 1 diabetes mellitus (T1DM) and hypoglycemia unawareness (*5–9*). There is no conclusive data currently available on the role of antibody-mediated rejection in the setting of allogeneic islet transplantation. Anti–human leukocyte antigen (HLA) antibodies can develop after exposure to allogeneic tissues through blood transfusion, pregnancy, or previous transplantation (*10*). The importance of alloantibody-mediated rejection has been recognized for solid organ transplants (*11*). Antibodies reacting with HLA expressed by kidney allografts can induce hyperacute and/or acute vascular rejection that frequently result in transplant failure (*12*). Scattered cases of HLA sensitization measured as positive panel reactive antibodies (PRA) preceding or associated with loss of islet graft function have been described in patients with T1DM bearing kidney allografts (islet after kidney [IAK] or simultaneous islet-kidney [SIK] transplantation) in the 1980s and 1990s (*13–15*). Recent data have shown that pretransplant PRA+ is associated with reduced survival of islet alone transplantation (ITA) (*16*). Interestingly, lack of allosensitization after ITA has been described, despite infusion of islets isolated from multiple donors (*5*, *8*, *17*), a phenomenon that may relate to efficacy of the new immunosuppressive regimens utilized (*5*, *7*, *8*, *17*). Loss of islet graft function may occur with the development of donor HLA sensitization (*18*), even in the absence of PRA+ in recipients of either ITA or IAK (*15*, *19*). Notably, IAK graft loss occurred without jeopardizing the function of the transplanted kidney (*15*, *19*). A positive association between allosensitization and islet graft loss (dysfunction or rejection) has been described in only few sequential ITA recipients (*17*, *20*, *21*), particularly in presensitized recipients (*21*).

MATERIALS AND METHODS

Patients

Sixty-six subjects with T1DM received allogeneic islet transplantation at the Diabetes Research Institute of the Uni-

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versity of Miami (UM) Miller School of Medicine between 1985 and 2006 as part of clinical trials active at the time of recruitment (Table 1). All trials were approved by the UM Institutional Review Board (IRB) and/or Western IRB (Olympia, WA). The trials have been grouped based on the era the transplants were performed, either before (Table 2) or after (Table 3) the year 2000. Inclusion criteria were similar among the trials: T1DM >5 years, age 18–65 years, negative C-peptide (<0.3 ng/ml by radioimmunoassay) in response to a mixed meal tolerance test (MMTT), and stable diabetes complications.

Islet Isolation and Transplantation

Islets were isolated using the automated method (*22*, *23*) and purification on discontinuous and/or continuous density gradients (*24*, *25*) from human pancreata obtained after cerebral death from heartbeating donors. Islets were transplanted by percutaneous transhepatic cannulation of the portal vein (*26*, *27*) or by access of a mesenteric tributary of the portal system after laparotomy (*28*).

Monitoring of Graft Function

Graft function was monitored by evaluating exogenous insulin requirements, glycated hemoglobin (A1c), and basal and stimulated C-peptide. Metabolic tests performed at baseline and follow-up included MMTT, intravenous glucose tolerance test, and intravenous arginine (*7*, *28*, *29*). Insulin independence was achieved when recipients were able to maintain A1c ≤6.5%, without exogenous insulin administration, with capillary fasting and postprandial blood glucose levels maintained at ≤7.8 mmol/L (140 mg/dL) and ≤10 mmol/L (180 mg/dL), respectively. Graft dysfunction was considered in C-peptide-positive recipients having fasting capillary glucose >7.8 mmol/L and/or postprandial capillary glucose >10.0 mmol/L in ≥3 occasions in 1 week, requiring reintroduction of exogenous insulin therapy, confirmed by a 90-min glucose level ≥10.0 mmol/L after MMTT (*7*, *29*). Graft failure was considered in patients with negative stimulated C-peptide (<0.3 ng/mL) during the follow-up and/or after discontinuation of immunosuppression (*29*, *30*).

Bone Marrow Cell Isolation

Bone marrow cells (BMC) were obtained from the vertebral bodies of the same donor of the islets (Table 1) in trials aiming at the induction of hematopoietic chimerism (*31–33*). In selected protocols, enrichment of CD34+ stem cells was obtained by positive selection using magnetic beads. The BMC inoculum was cryopreserved and then infused intravenously on days 5 and 11 after islet transplantation.

HLA Typing, Panel Reactive Antibodies, and Crossmatching

Complement-Dependent Cytotoxicity (CDC) Assay

The screening of sera for HLA-I and HLA-II antibodies was performed with a complement-dependent microlymphocytotoxic technique using a commercial kit (Lambda Cell Tray; OneLambda, Canoga Park, CA). Cross-matching of recipient serum with donor cells at the time of transplant was accomplished as previously reported (*34*).

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum PRA activity to HLA-I and HLA-II was determined using OneLambda antigen tray-mixed (LAT-M) standardized HLA-ELISA (OneLambda) (*35*).

FlowPRA and LABScreen Assays

Alloantibodies were detected by the means of flow cytometry techniques (Luminex Corporation, Austin, TX) using the fluorescent signal for each HLA-coated bead and normalized to the signal of negative control serum (*36*). The signal was considered positive when exceeding the cut-off value set by the manufacturer (OneLambda). The strength of a test sample was expressed by the fluorescence value. Percent positive of a test sample was calculated by dividing the number of positive beads by the total number of beads tested as per manufacturer's instructions (*36*).

Screening for HLA Antibodies and Donor-Recipient Matching Criteria

Donor and recipient HLA typing and cross-matching were tested prior to initiation of immunosuppression and/or islet transplant. A negative serum donor-recipient crossmatch and ABO compatibility was required at the time of transplant. All recipients were routinely tested for lymphocytotoxic PRA before and after transplantation, as well as after graft loss or immunosuppression discontinuation. Standard PRA screening was based on the CDC assay for all trials performed before the year 2000. In the present study, we have retested samples archived from patients in recent clinical trials using CDC, ELISA and flow cytometry. The cutoff for a PRA+ in the present study was >20%.

Donor-Specific and Nondonor-Specific Antibodies

Serum specificities were determined by CDC and ELISA using the LAT HLA-I and HLA-II reagents (OneLambda). The flow PRA tests can detect antibodies and their HLA specificities in each Labscreen panel. The single-antigen assay allows confirmation of antibody specificity suggested by a previous PRA test. Patients were considered PRA+ whenever antibodies against HLA-I and/or HLA-II donor-specific (DS) or not donor-specific (NDS) were detected by at least one test. It was presumed that subjects with HLA antibodies by ELISA/Luminex and negative CDC had complement-nonfixing antibodies, but they were equally considered as having DS or NDS antibodies (*37*).

Statistical Analysis

Data was analyzed using Microsoft Excel, Statistica 6.0 (StatSoft, Inc., Tulsa, OK), and SigmaPlot 9.0 (Systat Software, Inc.; San Jose, CA). Data are presented as means±standard deviation (SD). Statistical significance was considered for $P<0.05$.

RESULTS

Patients

Sufficient data on alloantibody monitoring was available for 66 recipients of islet allografts at our center between 1984 and 2006 (Table 2). Forty received ITA, 17 IAK, and 9 SIK transplants under the immunosuppressive protocols available in the different years (Table 1). Evaluation of PRA in

TABLE 1. Clinical trials

Groups	Year	Era	N	Type of transplant	BMC	Islet infusions per recipient (n)	Induction	Immunosuppression
1	1985–89	Before the year 2000	3 1	IAK SIK	No	1 2	—	Cyclosporine: 8–12 mg/kg (target trough levels 100–300 ng/mL), methylprednisolone: 1 mg/kg tapered within 1 wk to maintenance 0.1–0.2 mg/kg
2	1990–93		6 1	IAK SIK	No	1 (3) 2 (2) 3 (2)	Orthoclone OKT3: 5 mg IV daily for 14–16 days	Cyclosporine: dose adjusted to achieve whole blood trough levels of 250–300 ng/mL; methylprednisolone (MP): 500–1,000 mg MP daily for the first 3 d of OKT3 therapy. Thereafter IAK patients received their usual maintenance MP dose, and SIK patient was treated with higher doses of MP, following standard renal transplant protocols; azathioprine: 2 mg/kg
3	1994–96		7	SIK	Yes	1	ALS/ALG/Antithymoglobulin: 6 mg/kg IV for a total of 5 doses, on days −2,−1,0,+1, and +2 (n=3)	FK; mycophenolate mofetil: 500-1500 mg bid; Cyclosporine: dose adjusted to achieve whole blood trough levels of 250–300 ng/mL
			1	IAK			Orthoclone OKT3: 5 mg IV daily for 14–16 days (n=5)	Cyclosporine: dose adjusted to achieve whole blood trough levels of 250–300 ng/mL; azathioprine: dosage was adjusted according to white blood cell counts; tacrolimus
4	1998		5	I-BMT	Yes	1	Daclizumab (n=3) Antithymoglobulin: 6 mg/kg IV for a3 total of 5 doses, on days −2, −1, 0, +1, and +2 (n=2)	FK, MP, mycophenolate mofetil: 500-1500 mg bid (n=3) CSA, mycophenolate mofetil: 500-1500 mg bid (n=2)
5	2000	After the year 2000	6	I-BMT	CD34+	1	Daclizumab: 1 mg/Kg IV × 5 doses, first dose the day of transplant then every 14 days; infliximab: 5–10 mg/kg intravenously 2 h prior islet infusion	Sirolimus: 0.2 mg/kg orally pretransplant then 0.15 mg/kg once daily to attain 24-h trough levels of 12–15 ng/mL for the first 90 d, and 10–12 ng/mL thereafter; Tacrolimus: 1 mg twice daily to maintain 12-h trough levels of 3–6 ng/mL
6	2001		19	ITA	No	1 (5) 2 (10) 3 (4)	Daclizumab: administered also bimonthly during the second year; infliximab: 5–10 mg/kg intravenously 2 h prior islet infusion	Sirolimus: 0.2 mg/kg orally pretransplant then 0.15 mg/kg once daily to attain 24-h trough levels of 12–15 ng/mL for the first 90 d, and 10–12 ng/mL thereafter; Tacrolimus: 1 mg twice daily to maintain 12-h trough levels of 3–6 ng/mL
7	2002–05		7	IAK	No	1 (2) 2 (5)	Daclizumab: administered also bimonthly during the second year; Infliximab/Etanercept: 50 mg IV 1 h prior to transplant, followed by 25 mg subcutaneously twice a week for 2 wk	Sirolimus: 0.2 mg/kg orally pretransplant then 0.15 mg/kg once daily to attain 24-h trough levels of 12–15 ng/mL for the first 90 d, and 10–12 ng/mL thereafter; Tacrolimus: 1 mg twice daily to maintain 12-h trough levels of 3–6 ng/mL

TABLE 1. Continued

Groups	Year	Era	N	Type of transplant	BMC	Islet infusions per recipient (n)	Induction	Immunosuppression
8	2002		5	ITA	No	1 (1) 2 (3) 3 (1)	Daclizumab: administered also bimonthly during the second year; Infliximab/Etanercept: 50 mg IV 1 h prior to transplant, followed by 25 mg subcutaneously twice a week for 2 wk	Sirolimus: 0.2 mg/kg orally pretransplant then 0.15 mg/kg once daily to attain 24-h trough levels of 12–15 ng/mL for the first 90 d, and 10–12 ng/mL thereafter; Tacrolimus: 1 mg twice daily to maintain 12-h trough levels of 3–6 ng/mL
9	2005		2	I-BMT	CD34+	1	Campath-1H: 20 mg IV over 3 h given on day −1 and a second dose of 20 mg IV administered on the day of transplant	Sirolimus: 0.2 mg/kg orally pretransplant then 0.15 mg/kg once daily to attain 24-h trough levels of 12–15 ng/mL for the first 90 d, and 10–12 ng/mL thereafter; Tacrolimus: 1 mg twice daily to maintain 12-h trough levels of 3–6 ng/mL; Mycophenolate mofetil: introduced after 3 months to replace tacrolimus.
10	2005–06		3	ITA	No	1 (2) 2 (1)	Campath-1H: 20 mg IV over 3 h given on day −1 and a second dose of 20 mg IV administered on the day of transplant	Sirolimus: 0.2 mg/kg orally pretransplant then 0.15 mg/kg once daily to attain 24-h trough levels of 12–15 ng/mL for the first 90 d, and 10–12 ng/mL thereafter; Tacrolimus: 1 mg twice daily to maintain 12-h trough levels of 3–6 ng/mL; Mycophenolate mofetil: introduced after 3 months to replace tacrolimus.

ALG, antilymphocyte globulins; ATG: antithymoglobulin; ALS, antilymphocyte serum; BMC, bone marrow cells; IAK, islet after kidney; I-BMT, islet-bone marrow transplantation; ITA, islet transplantation alone; SIK, simultaneous islet-kidney.

TABLE 2. Patient demographics and HLA

Subject	Protocol: Trial	Protocol: Type of transplant	Age at transplant	Sex (M/F)	P_n,D_n,M_n	T1DM duration (years)	IEQ/kg	No. of islet donors	Insulin independence duration, days (postsupplemental infusion)	Islet graft failure (POD)	Mismatches of islet donors: A	B	DR
1	1985–89	IAK	39	M	—	34	NA	1	Not achieved	13	11	35,40	6,9
2	1985–89	SIK	25	F		23	NA	1	Not achieved	51	3,24	7,16	1
3	1985–89	IAK	35	F		26	NA	2	Not achieved	338	24	7,15	1,2
4	1985–89	IAK	42	F		23	NA	1	Not achieved	30	2,24	44,50	-
5	1990–93	IAK	36	F		19	8,208	5	Not achieved	43	1,2,3,26,28,30	4,7,8,42,44,65	2,6,7,8,10,12
6	1990–93	IAK	40	F		24	8,318	1	Not achieved	360	2,3	7,39,64	1,2,7
7	1990–93	IAK	31	M	—	24	10,574	2	Not achieved	>5,023	1,2,36	7,8,53	1,2,5,9
8	1990–93	SIK	37	F		19	12,752	3	Not achieved	756	1,3,31	5,7,8,13,35	2,5,6
9	1990–93	IAK	36	F		20	17,951	3	Not achieved	>4,492	2,32	5,14,16,39,60	5,6,9
10	1990–93	IAK	29	F		20	18,884	3	Not achieved	154	1,3,11,19,24,29	7,35	1,2,3
11	1990–93	IAK	36	M	—	18	18,699	3	Not achieved	307	1,26,33,34	7,8,57,70	3,5,6,8
12	1994–96	SIK-BMT	35	M	—	26	14,080	1	Not achieved	64	2	39	4
13	1994–96	IAK-BMT	48	F		40	11,838	1	Not achieved	184	24	14,39	7,11
14	1994–96	SIK-BMT	46	F		28	15,691	1	Not achieved	119	2,11	27,51	14
15	1994–96	SIK-BMT	32	M	—	20	5,786	1	Not achieved	168	1,28	27,37	10
16	1994–96	SIK-BMT	36	F		17	22,934	2	Not achieved	229	24,31	17,27,55,62	2,13
17	1994–96	SIK-BMT	45	F		32	26,349	2	Not achieved	91	25,28	53,62,70	7
18	1994–96	SIK-BMT	25	M	—	19	15,120	1	Not achieved	142	28	57,60	10
19	1994–96	SIK-BMT	54	F		31	6,583	1	Not achieved	167	-	7,60	13
20	1998	I-BMT	40	M	—	13	7,837	1	Not achieved	21	2,11	44,52	11,15
21	1998	I-BMT	42	F		31	9,733	1	Not achieved	130	1,24	35,38	11,12
22	1998	I-BMT	46	M	—	25	6,768	1	Not achieved	16	26	35,51	4,11
23	1998	I-BMT	39	F		14	10,587	1	Not achieved	45	1,34	41	-
24	1998	I-BMT	41	F		34	6,722	1	Not achieved	494	2,28	49,51	11
25	2000	I-BMT	40	M	—	15	7,046	1	Not achieved	452	11	44	13,15
26	2000	I-BMT	39	F	P_0,D_0,M_0	34	12,485	1	50	479	3,24	51	16,17
27	2000	I-BMT	45	F	P_2,D_2,M_0	26	8,012	1	Not achieved	178	30,31	53,57	11,13
28	2000	I-BMT	44	F	P_3,D_2,M_1	40	7,039	1	18	471	3	44,50	1,7
29	2000	I-BMT	42	F	P_1,D_1,M_0	41	7,534	1	Not achieved	158	3	27,44	8,11
30	2000	I-BMT	26	M	—	14	9,547	1	Not achieved	510	24	35,45	4,17
31	2001	ITA	36	M	—	10	29,593	4	256	1,647	3,24,32,74	18,21,39,44,52,63,70	2,7,8,11,13
32	2001	ITA	29	F	P_0,D_0,M_0	20	13,122	1	Not completed	72	2,3;	—	11,13
33	2001	ITA	41	F	P_2,D_2,M_0	31	13,421	2	380	836	3,32	44,62	7,13,14,15
34	2001	ITA	32	F	P_0,D_0,M_0	19	13,324	4	1196	>2,022	2,23,26,31	14,38,39,44,51,70	1,7,13,15
35	2001	ITA	38	M	—	14	12,956	2	1455	1,715	1,2,3,24	7,35,57,60	6,11,15
36	2001	ITA	43	F	P_0,D_0,M_0	37	27,584	3	185 (175)	1,458	24,68	47,61,62,81	1,7,8,11,14

TABLE 2. Continued

Subject	Protocol		Age at transplant	Sex (M/F)	P_n,D_n,M_n	T1DM duration (years)	IEQ/kg	No. of islet donors	Insulin independence duration, days (postsupplemental infusion)	Islet graft failure (POD)	Mismatches of islet donors		
	Trial	Type of transplant									A	B	DR
37	2001	ITA	44	F	P_1,D_1,M_0	31	19,72	3	397 (303)	>1,956	3,28,32	38,51,62	4,9,11,13,15
38	2001	ITA	36	M	—	34	25,282	3	470 (>1499)	>1,916	2,3	7,18,44,62,72	1.17
39	2001	ITA	35	M	—	17	6,251	3	968 (>462)	>1,904	2,23,31	6,44	1,11,13
40	2001	ITA	37	M	—	35	15,59	2	453	755	3,28,30	13,18,44,62	4,17
41	2001	ITA	25	F	P_2,D_2,M_0	9	5,262	1	Not completed	364	11,31	51	13
42	2001	ITA	52	M	—	40	9,823	3	1307	>1,809	25	7,27	1
43	2001	ITA	60	M	—	47	19,56	3	166 (109)	>1,762	1,2,28,29,33	17,18,35,44	1,2,3,7,11
44	2001	ITA	53	F	P_1,D_0,M_1	45	19,746	4	181	898	1,24	35,38,57	7,11,13
45	2001	ITA	56	F	P_1,D_0,M_1	35	13,288	2	400	>1,675	1,38	8,18,27,62	3,4,8,17
46	2001	ITA	41	F	P_3,D_2,M_1	12	13,185	1	795	1,182	25	6,18	15,16
47	2001	ITA	47	F	P_3,D_3,M_0	13	6,311	1	Not achieved	>251	—	62,65	15,4
48	2001	ITA	65	M	—	15	5,249	2	88	>480	2,29	44,45,49,62	4,13,15
49	2001	ITA	45	F	P_5,D_2,M_3	38	11,779	1	>640	>640	23,3	7,44	8,15
50	2002	ITA	35	F	P_3,D_0,M_3	27	9,866	3	963	>1,686	1,68	—	11
51	2002	ITA	48	M	—	45	17,178	3	642 (>437)	>1,595	2,29	4,50,51,62	1,4,17
52	2002	ITA	35	F	P_3,D_2,M_1	19	13,911	2	626 (>471)	>1,562	2,3,28	7,38,49,58	7,13,15
53	2002	ITA	51	F	P_2,D_1,M_1	21	7,681	1	713 (>266)	>1,552	1,25	18	15
54	2002	ITA	33	M	—	11	13,206	2	Not achieved	497	26	7,35,44	11
55	2002–05	IAK	49	M	—	42	8,470	1	280	>1,531	3	60	15
56	2002–05	IAK	35	F	P_0,D_0,M_0	29	16,356	3	413	>1,440	2,24,31	16,27,35,39	1,3,15
57	2002–05	IAK	51	M	—	34	15,356	2	531	>1,411	3	18,44,62	4,7,11
58	2002–05	IAK	42	F	P_0,D_0,M_0	47	20,189	2	211	>1,060	2,23,25	7,18,41	11,15
59	2002–05	IAK	48	F	P_1,D_0,M_1	37	17,139	3	20	>918	11,24	7,35,47	1,7,9,15,103
60	2002–05	IAK	43	M	—	39	14,028	1	135	284	2	14,57	7,9
61	2002–05	IAK	44	M	—	34	11,874	2	Not achieved	>715	23,30,33,36	7,42,53,57	9,13,18
62	2005	I-BMT	49	F	P_2,D_0,M_2	43	5,792	3	Not achieved	>311	28	44	9,11
63	2005	I-BMT	44	F	P_1,D_1,M_0	29	7,864	1	279	>547	24,25	27,98	4
64	2005–06	ITA	43	F	P_1,D_0,M_1	42	7,028	1	>755	>755	36,68	35	8.15
65	2005–06	ITA	42	F	P_0,D_0,M_0	29	13,526	2	>746	>746	1,2,29,32	7,8,60	15.17
66	2005–06	ITA	38	F	P_4,D_3,M_1	20	4,945	1	Not achieved	>354	2,68	7,42	13,18

PnDnMn: number of pregancies (P), deliveries (D), and miscarriages (M).

TABLE 3. PRA values and clinical outcome in allosensitized subjects before or during follow-up

Subject	Protocol: Trial	Protocol: Type of transplant	PRA method	PRA pretransplant: HLA-I	PRA pretransplant: HLA-II	PRA on immunosuppression: HLA-I	PRA on immunosuppression: HLA-II	PRA off immunosuppression: HLA-I	PRA off immunosuppression: HLA-II	Islet graft failure (day)
2	1985–89	SIK	CDC	32%	NA	8%	NA	On immunosuppression for kidney graft		13
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
4	1985–89	IAK	CDC	41%	NA	36%	NA	On immunosuppression for kidney graft		30
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
6	1990–93	IAK	CDC	31%	30%	48%	71%	On immunosuppression for kidney graft		360
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
7	1990–93	IAK	CDC	0%	4%	0%	29%	On immunosuppression for kidney graft		>5,023
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
8	1990–93	SIK	CDC	20%	7%	5%	4%	On immunosuppression for kidney graft		756
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
9	1990–93	IAK	CDC	0%	3%	10%	34%	0%[b]	44%[b]	>4,492
			ELISA	NA	NA	NA	0%	NA	NA	
			Flow	NA	NA	NA	0%	NA	NA	
10	1990–93	IAK	CDC	8%	0%	75%	27%	On immunosuppression for kidney graft		154
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
11	1990–93	IAK	CDC	28%	23%	31%	11%	On immunosuppression for kidney graft		307
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
14	1994–96	SIK-BMT	CDC	31%	57%	2%	11%	On immunosuppression for kidney graft		119
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
15	1994–96	SIK-BMT	CDC	28%	11%	0%	6%	On immunosuppression for kidney graft		168
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
16	1994–96	SIK-BMT	CDC	24%	39%	45%	64%	On immunosuppression for kidney graft		229
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
17	1994–96	SIK-BMT	CDC	2%	32%	2%	18%	On immunosuppression for kidney graft		91
			ELISA	NA	NA	NA	NA			
			Flow	NA	NA	NA	NA			
18	1994–96	SIK-BMT	CDC	0%	0%	43%	14%	36%	21%	142
			ELISA	NA	NA	NA	NA	NA	NA	
			Flow	NA	NA	NA	NA	NA	NA	
22	1998	I-BMT	CDC	0%	28%	NA	NA	0%	0%	16
			ELISA	NA	NA	NA	NA	NA	NA	
			Flow	NA	NA	NA	NA	NA	NA	
23	1998	I-BMT	CDC	50%[a]	0%	69%	64%	0%	0%	45
			ELISA	80%	0%	NA	NA	NA	NA	
			Flow	87%	0%	NA	NA	NA	NA	
27	2000	I-BMT	CDC	0%	0%	21%[a]	NA	0%	NA	178
			ELISA	29%	6%	20%	25%	95%	99%	
			Flow	36%	29%	31%	46%	84%	97%	

TABLE 3. Continued

Subject	Protocol: Trial	Protocol: Type of transplant	PRA method	PRA pretransplant: HLA-I	PRA pretransplant: HLA-II	PRA on immunosuppression: HLA-I	PRA on immunosuppression: HLA-II	PRA off immunosuppression: HLA-I	PRA off immunosuppression: HLA-II	Islet graft failure (day)
	2000	I-BMT	CDC	21%[a]	NA	0%	NA	22%	NA	471
			ELISA	80%	41%	79%	56%	79%	72%	
			Flow	73%	40%	84%	77%	80%	86%	
29	2000	I-BMT	CDC	18%	0%	46%[a]	0%	0%	NA	158
			ELISA	54%	0%	84%	0%	91%	38%	
			Flow	56%	0%	44%	0%	84%	43%	
46	2001	ITA	CDC	2%	0%	0%	0%	0%	0%	1,182
			ELISA	0%	0%	0%	0%	11%	0%	
			Flow	0%	26%	0%	26%	36%	20%	
53	2002	ITA	CDC	0%	0%	0%	0%	Ongoing immunosuppression		>1,552
			ELISA	0%	0%	0%	0%			
			Flow	0%	0%	31%	0%			
57	2002–05	IAK	CDC	5%	0%	34%	63%	Ongoing immunosuppression		>1,411
			ELISA	NA	NA	38%	41%			
			Flow	NA	NA	29%	43%			
60	2002–05	IAK	CDC	0%	0%	39%	NA	Ongoing immunosuppression		284
			ELISA	0%	0%	20%	13%			
			Flow	NA	NA	27%	34%			

[a] Positive PRA by CDC obtained at the reanalysis in patient who was considered negative at the time of first testing before transplant.
[b] Immunosuppression (IS) had been partially discontinued. Azathioprine and cyclopsporine had been stopped. Methylprednisolone was continued.

the trials performed before the year 2000 (Table 2) was based on CDC.

HLA Phenotype of Islet Allograft Recipients and Their Donors

Donor and recipient HLA-I and HLA-II phenotypes were retrospectively analyzed and were not considered as a determinant for transplant. Overall, HLA-A, -B, and -DR matching as well as HLA phenotype of islet allograft recipients is presented for trials performed before and after the year 2000 (Table 2). All grafts had ≥2 mismatches with the recipient and very few alleles were shared between first and subsequent transplants. In particular, only two subjects (4 and 23) shared the same HLA-II with the donor, while none shared HLA-I haplotypes (Table 2). The mean number of HLA mismatches was 7.1±3.3 (HLA-I: 4.8±2.3; HLA-II: 2.3±1.3).

HLA Antibodies Before Islet Transplantation

All 66 subjects were crossmatched negative before islet transplantation; 56 were also PRA-negative by CDC. Of the 10 subjects with a PRA+ within the 3 months preceding islet infusion, six were SIK (2, 8, 14, 15, 16, and 17), three were IAK (4, 6, and 11), and one was ITA (22) (Table 3). Subject 22 was PRA+ only for HLA-II (28%). Islet graft function was variable (range 21–360 days) in these patients. Subject 4 had graft function for only 1 month. Subject 6 had an allogeneic kidney transplanted for end-stage renal disease (ESRD) 6 years earlier and, despite PRA+ at baseline (HLA-I=31%, HLA-II=30%), maintained islet graft function up to 360 days. Stable kidney function was observed despite persistence of PRA+ for HLA-I (islet DSA: A2 and B39) (Table 4) and HLA-II (range 68–82%) throughout the follow-up as well as after islet graft loss. Subject 11 had PRA+ at baseline (HLA-I: 31%; HLA-II: 18%), received 3 allogeneic islet preparations, and tested negative until 9 months after the first islet infusion (HLA-I: 31%; HLA-II: 11%; DSA: B8, B57, DR6 and DR8) (Table 4). One month after allosensitization, C-peptide was no longer detectable, while kidney function remained stable. Subject 16 displayed PRA+ 1 and 6 months after transplantation. While C-peptide was no longer detectable 2 months later (postoperative day, POD 229), kidney graft function remained unchanged and at the most recent follow-up (3 years after islet failure) remains PRA-negative.

Trials performed before the year 2000 were solely based on PRA assessed by CDC, while ELISA and/or flow cytometry were introduced afterwards. Depending on archived specimen availability, sera from selected patients were re-tested to evaluate whether graft outcomes could be attributed to undiagnosed allosensitization by CDC at the time of transplant (Table 3). This re-analysis identified six additional subjects who previously tested negative by CDC at the time of transplant (subjects 23, 27, 28, 29, 46, and 53), all women with previous pregnancies (range 1–4; Table 2). Subject 23 discontinued immunosuppression due to nonadherence to protocol and islet graft function was lost at POD 45 (*30*). Islet graft survival for subjects 27 and 29 was 164 and 158 days, respectively, which was shorter than that of other patients in the same trial (I-BMT; mean graft survival 374.7±161.3 days in this trial). Subject 28 had showed islet allograft function for 471 days, a time similar to that of other patients in the same

TABLE 4. Development of donor-specific and nondonor-specific antibodies in recipients of ITA and I-BMT after the year 2000

Subject	No. of donors	Test	No. of donor-specific antibodies to islet donor 1	2	3	4	Nondonor-specific antibodies
22	1	Baseline	DR11[a]	NA	NA	NA	—
23	1		B41	NA	NA	NA	—
27	1		—	NA	NA	NA	A1,23,32,36,80; B7,8,13,18,27,37,42,47,49,50,59,60; DR1,10,15,16
28	1		B44[d] DR1[d]	NA	NA	NA	A1,23,24,25,29,32,33,34,66,80; B8,13,27,37,45,47,49,51,52,53,57,58,59,63,64,65; DR7,9,10,15,16,51,53
29	1		—	NA	NA	NA	A2,11,24,25,26,29,30,34,66; B8,38,39,48,51,52,54,55,56,59,60,64,65,67,72,81
42	2		DR13	A1 DR17	NA	NA	**A29; B76** (patient still on IS); DR8,16
63	1		A25	NA	NA	NA	A80; B13,50
23	1	On	—	NA	NA	NA	A1,3,30,31; B7,8,13,18,27,35,42,48,50,54,55,56,B59,60,61,62,65,67,71,72,75,76,81,82; DR52
25	1		B44	NA	NA	NA	NA
27	1		A31	NA	NA	NA	A26,31,32; B7,13,41,47,49,50,52,60,61,67,72; DR1,4,6,10,15,16
28	1		B44 DR1	NA	NA	NA	A1,11,23,24,25,26,32,80; B13,27,37, 41,45,47,49,51,52,53,57,58,59,60,61,63; DR4,10,15,16,51
29	1		A3 B44	NA	NA	NA	A11,23,24,26,32,34,36,66,68; B7,37,41,45,47,53,54,59,60,81
21	1	Off	A1,24 B38 DR11	NA	NA	NA	A3,11,23,25,32,33,36,68,80; B8,13,27,37,38,44,47,**48**,49,51,52,53,57,58,59,63,64,65; DR11,16,52[b]
23	1		A1	NA	NA	NA	A1,A30,31,80; B59; DR52
24	1		B49,51	NA	NA	NA	A1,2,11,23,24,25,32,80; B8,13,27,37,38,44,47,52,53,57,58,59,63,64,65; DR8,**14**[c]
25	1		A11 B44 DR13,15	NA	NA	NA	A23,24,25,32,66,80; B8,13,26,27,37,45,47,49,51,52,53,57,58,59,60,61,63,64; DR1,8,11,12,16,51
26	1		A3,24 B51 DR16	NA	NA	NA	A23,25,32; B13,27,37,41,44,47,49,52,53,57,58,59,63; DR1,**11**,12,13,15,51
27	1		A30,31 B53,57 DR11,13	NA	NA	NA	A1,23,24,25,32,36,80; B13,27,35,37,44,47,49,51,52,58,59,63,64,65; DR1,**4**,8,10,12,15 ,16,51,52,53
28	1		B44 DR1,7	NA	NA	NA	A1,11,23,24,25,26,32,34,80; B13,27,33,37,45,47,49,51,52,53,58,57,59,60,61,63; DR4,9,10,15,16,51,53
29	1		A3 B27,44 DR8,11	NA	NA	NA	A1,23,24,25,32,36,80; B8,13,35,37,45,47,51,52,53,57,58,59,62,63,64,65,75,76; DR7,11,12,15,16,51
30	1		A24 B35 DR4	NA	NA	NA	A1,23,25,32,34,66; B41,46,49,50,51,52,53,57,58,63,72; DR1,8,10,11,12,13,51,53
31	4		A3 DR13	DR8,11	A24,32 B52 DR15	A3 DR13	A1,11,23,25,26,29,30,36,74,80; B7,8,13,18,27,37,41,46,47,49,51,53,54,55,56,58,59,60,63,65,78; DR16,10

TABLE 4. Continued

Subject	No. of donors	Test	No. of donor-specific antibodies to islet donor 1	2	3	4	Nondonor-specific antibodies
35	2		A3,24	A1,2	NA	NA	A11,23,36,68,69,80; B37; DR8
40	2		—	A3	NA	NA	A1,11,24,25,26,32,33,34,36,66,68,69,80,81; B8,58; DR8,11,12,13,51,52
41	1		A11,31 B51 DR4	NA	NA	NA	A1,23,24,25,26,30,32,36,66; B7,8,27,35,42,44,45,52,53,57,58,59,61,62,63,64,71,72,75,76; DR1,5,11
44	3		A1,24 DR7	A1 B35,57 DR7	B57	A1	A3,11,23,25,32,36,80; B8,13,27, 47,49,50,51,52,53, 58,59,62,63,64,65,69,71,72,75; DR9
54	2		—	B44 DR11	NA	NA	A1,29; B13,27,37,45,47,76,79; DR8,11,12,13,51,52,53

Boldface indicates that DSA and NDSA were present also during IS therapy but not at baseline.
[a] Flow/ELISA PRA performed only on baseline.
[b] Flow/ELISA PRA performed only off IS.
[c] Flow PRA performed on and off IS, not on baseline.
[d] DSA present also during IS and after IS withdrawal.
DSA, donor-specific antibodies; IS, immunosuppression; NA, not available; NDSA, nondonor-specific antibodies.

trial (479, 452, and 510 days, respectively) who had immunosuppression discontinued per protocol. After year 2000, two ITA recipients (subjects 46 and 53) with history of 3 and 2 pregnancies, respectively, displayed a PRA+ by Flow (subject 46: HLA-I=0%, HLA-II=26%; subject 53: HLA-I=29%, HLA-II=0%), while being negative by CDC and ELISA (Table 2). Subject 46 was withdrawn from the study (POD 1182) after development of adenoviral encephalitis requiring hospitalization and subsequent discontinuation of immunosuppression. Subject 53 achieved and sustained insulin independence for 714 days after a single donor islet infusion. She received a supplemental islet infusion on POD 1221 and maintains PRA-negative by ELISA at the most recent follow-up (POD 1552).

Evaluation of the impact of pretransplant HLA sensitization on the outcome of islet grafts performed either before and after 2000 showed no significant differences when comparing patients with PRA positive (>20%) and negative (<20%) pretransplant (Fig. 1A). A trend (although not statistically significant) toward a positive correlation of PRA+ pretransplant and duration of graft function was observed in the patients of trials performed after 2000, although the small number of PRA+ patients at baseline (n=3) may account for this observation. Similarly, the impact of PRA+ after islet transplantation on graft function showed unremarkable differences in study subjects of trials performed before and after 2000 (Fig. 1B). Interestingly, in the trials performed before 2000, two patients with PRA+ maintained long-term graft function (Fig. 1B).

HLA Antibodies During Follow-Up

Allosensitization Status by CDC

Of the 66 study subjects (all years), only 13 (19.6%) displayed PRA+ during follow-up. Eight (12%) of them developed PRA+ de novo and five had PRA+ pretransplant (Table 3). No other patients developed a PRA+ by CDC at any time during the follow-up while on immunosuppression, despite graft loss/dysfunction occurring in some cases (n=28). Three of the patients who developed PRA+ while on immunosuppression (subject 7, 9, and 57) have enjoyed long-term islet graft function. Subject 7 maintained islet graft function for >13 years (*28*, *30*) and developed PRA+ 3 years after islet transplant (DSA: A1, A23, B7, B8 and DR9 against both islet donors; Tables 3 and 4). Subject 9 received three islet preparations 3 years after a kidney allograft and developed DSA against the three islet donors (DR5, DR6, and DR9) 180 days after transplantation (Table 4). Islet function persisted for >13 years (*28*, *30*). Kidney rejection occurred 11 years after IAK; therefore azathioprine and cyclosporine were discontinued, while maintaining methylprednisolone (MP). Sustained C-peptide was detectable until death at POD 4,492. One year after kidney rejection, sensitization occurred only for HLA-II (range 32–44% by CDC), despite MP maintenance. Subject 57 received two sequential islet infusions 12 years after an allogeneic kidney (2002–05 trial). Forty-two months after the second islet infusion, he displayed PRA+ by CDC (HLA-I: 34%; HLA-II: 63%), which coincided with an EBV infection. Two months before developing PRA+, MP was tapered and discontinued. At the present time (>2 months from PRA+ detection), stable islet and kidney graft function is maintained under target trough levels of immunosuppression.

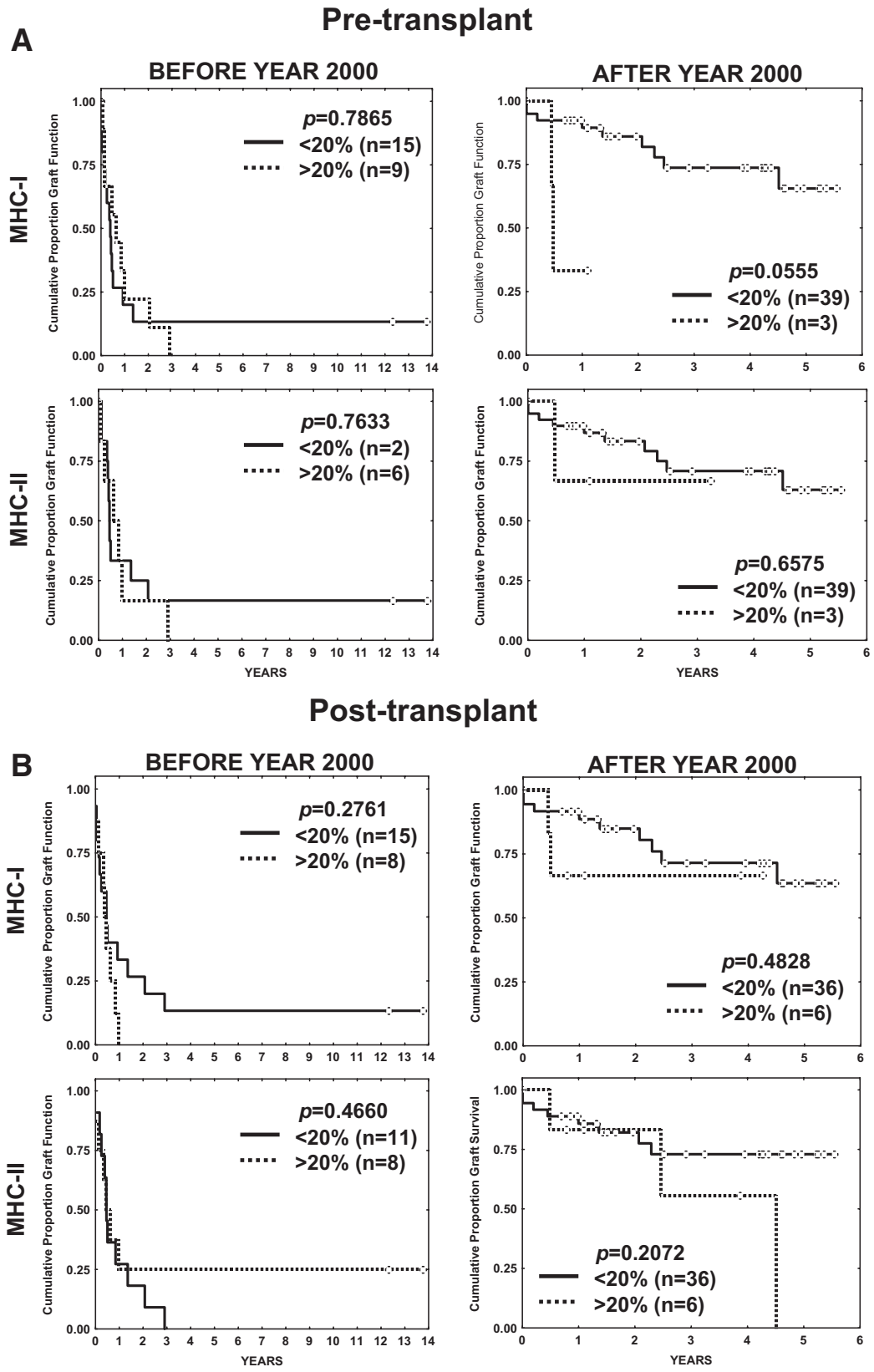

FIGURE 1. Evaluation of PRA status on islet allograft function in the trials performed before (left panels) and after the year 2000 (right panels). (A) Effects of HLA status pretransplant (PRA+: >20%, dotted lines; PRA-: <20%, solid lines). (B) Effects of HLA status any time posttransplant.

In the seven SIK-BMC transplants (1994–96 trial; Table 1), overall islet graft function was 140±55 days (range 64–229). Subject 18 developed PRA+ by CDC 1 month after SIK transplantation (HLA-I: 98%; HLA-II: 18%). After hepatitis C diagnosis, interferon (IFN)-α treatment was implemented at POD 45, resulting in prompt rejection of both islet and kidney grafts (*30*). Notably, kidney allograft survival in the patients in this trial was >600±170 days with well-preserved renal function (creatinine 0.3 mg/dl, n=6) and with no episodes of renal rejection at the follow up, despite loss of C-peptide.

Subject 60 (2002–05 IAK trial) received a single islet infusion 4.5 years after kidney allotransplantation. Due to severe adverse events (pneumonia and severe skin lesions) requiring hospitalization 6 months after islet implantation, sirolimus trough levels were reduced, resulting in resolution of the complications. Development of PRA+ for HLA-I and subsequent loss of islet graft function were recorded soon after (POD 284), while kidney graft function was maintained. Subjects 27 and 29 were discussed earlier, with islet graft function of 178 and 158 days, respectively. Subject 10 (1990–93) showed graft function for 154 days (Table 3).

Different immunosuppressive regimens have been utilized in the different trials considered in this study. We have therefore grouped the trials based on the time of introduction of more powerful immunosuppression based on sirolimus-tacrolimus maintenance in the year 2000 (*5*, *7*, *30*). In the trials before 2000, 37.5% (9/24) of subjects displayed PRA+ by CDC while on immunosuppression (subjects 4, 6, 7, 9, 10, 11, 16, 18, and 23; Table 3). By contrast, only 9.5% (4/42) of patients after 2000 displayed PRA+ by CDC while on immunosuppression (subjects 27, 29, 57, and 60; Table 2); only 7/42 subjects were IAK, with the majority being ITA (n=27) and I-BMT (n=8). Notably, in trials performed before 2000, the majority of the cases were IAK (n=10) or SIK (n=9).

For 41 patients transplanted after year 2000, PRA data assessed by CDC, ELISA, and Flow while on immunosuppression was available. All had persistent C-peptides, but required reintroduction of exogenous insulin after graft dysfunction (*7*). Seven (17%) displayed PRA+ by any of the different tests while on immunosuppression (subjects 27, 28, 29, 46, 53, 57, and 60; Table 3). Only three of them (7%) developed PRA+ de novo (subjects 53, 57, and 60, discussed earlier), while the others were positive pretransplant.

HLA Sensitization After Immunosuppression Withdrawal

Retesting of sera specimens from a total of 35 patients participating in 1998 trials of ITA and I-BMT was performed using a combination of CDC, ELISA, and Flow PRA. Thirty-two subjects (91%) were PRA-negative while on immunosuppression based on any of the methods (mean follow-up 708±581 days). A total of 16 subjects discontinued immunosuppression and withdrew from the trials (per protocol, n=4; voluntary, n=6; or due to adverse events, n=6) (*7*, *30*, *38*). Sufficient serum samples for reanalysis were available for 14/16 patients (ELISA and Flow; CDC in only 12 subjects; Fig. 2). Analysis of PRA in this group was performed at baseline, after transplantation while under treatment with immuno-

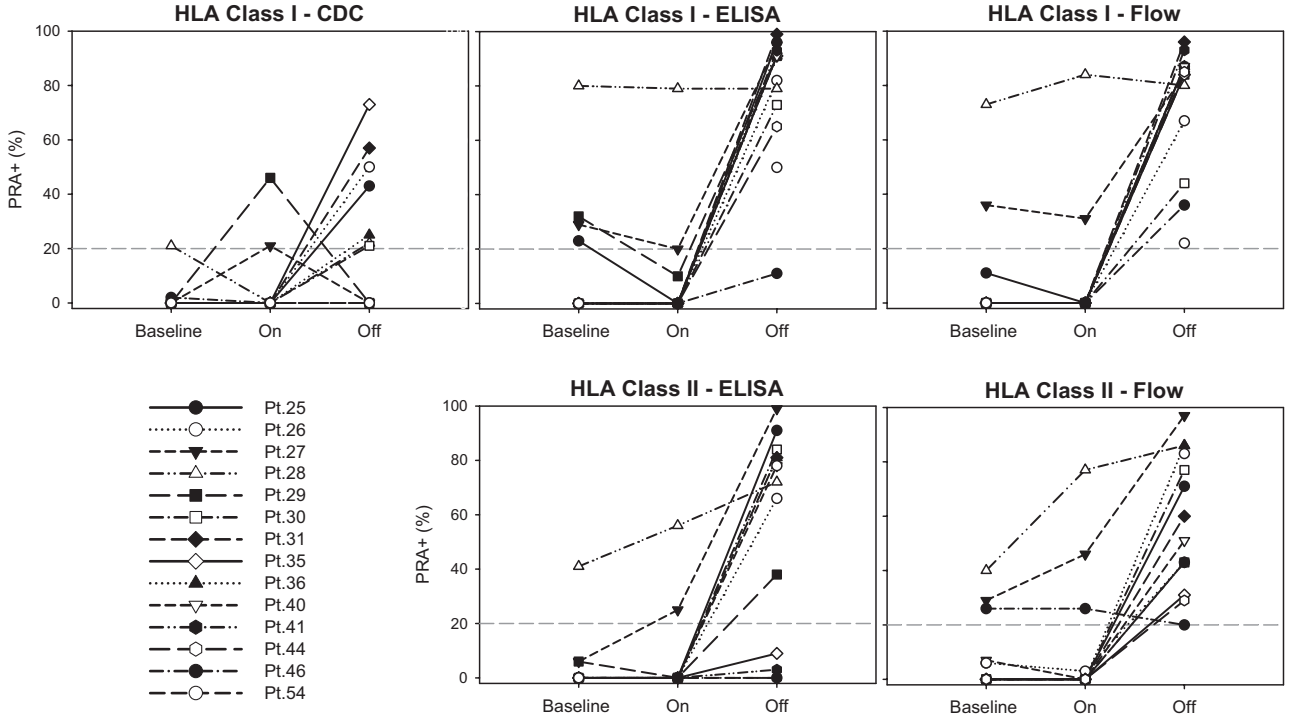

FIGURE 2. Evaluation of PRA in 14 patients in whom immunosuppression was discontinued (all from 2000) receiving ITA or I-BMT by CDC, ELISA, and Flow. Monitoring of PRA was performed before islet transplantation (baseline), while under immunosuppression (on), and after discontinuation of immunosuppressive drugs (off). Alloantibody anti HLA-I (upper panels) and HLA-II (lower panels) are shown. The horizontal broken indicate the cutoff for positive PRA (>20%) in all panels. The number in the legend refers to patient identification.

suppressive drugs (on), and after immunosuppression withdrawal (off).

PRA-negative by CDC was recorded at the time of transplant in all 16 subjects. Retesting of specimens from 14 of these subjects showed that one subject (28) was PRA+ by CDC (HLA-I: 21%), ELISA (HLA-I: 80%; HLA-II 42%), and Flow (HLA-I: 73%; HLA-II 40%; Figure 2).

During follow-up and while on immunosuppression, only three subjects were PRA+ (subjects 27, 28, and 29; Fig. 2). Data for PRA by CDC was available for only 12 patients. Subject 27 displayed PRA+ in the borderline values by CDC (HLA-I: 21%) and ELISA (HLA-I: 20%) with frankly positive Flow (HLA-I: 31%). Subject 28 was PRA-negative by CDC but frankly positive by ELISA (HLA-I: 79%; HLA-II: 56%) and Flow (HLA-I: 84%; HLA-II: 77%). Subject 29 displayed PRA+ by CDC (HLA-I: 46%) that was not confirmed by ELISA and Flow (HLA-I: 10% and 0%, respectively) on the same specimen (Fig. 2).

After drug withdrawal (POD 133±68, range 29–287 days), PRA+ for HLA-I by CDC was observed in 58.3% (7/12) of patients for whom data was available (HLA-I: 24.25±25.9%), while 41.6% (n=5) remained PRA-negative (Figure 2). Evaluation of PRA by ELISA and Flow showed that all 14 patients for whom data was available were positive (>20% PRA) for both HLA-I (ELISA: 79.3±23.9%; Flow: 74±23%) and HLA-II (ELISA: 55.7±37.2%; Flow: 58.6±24.8; Figure 2).

Development of Donor-Specific Antibody

When evaluating only the recipients of ITA and I-BMT performed after 1998 (n=35; Table 2), we have identified five subjects (22, 23, 28, 42, and 63) with donor-specific antibody (DSA) positive before islet transplantation all of which were negative while on immunosuppressive treatment, with only one exception (subject 23; Table 4). Subject 28 presented with DSA again after discontinuation of immunosuppressive therapy (Table 4). Only subjects 23 and 25 had DSA during follow-up while on immunosuppression (Table 4). Development of DSA to ≥1 donor HLA occurred in 75% (12/16) of the patients after discontinuation of immunosuppression (Table 4). Development of alloantibody against HLA not specific for any of the islet donors was also detected in these patients (Table 4).

DISCUSSION

The deleterious impact of PRA+ at the time of transplant on the outcome of solid organ grafts has been recognized (*10*). Similar observations have been reported in the setting of islet transplantation (*13*, *14*, *16*, *19*, *20*). Collectively, even though functional impairment of the islet allograft was observed in some cases of PRA+ recipients (*8*, *21*), a cause-relationship could not be formally established on a large number of observations thus far (*18*).

In the present study, we have retrospectively evaluated the development of alloantibodies in patients with T1DM who underwent allogeneic islet transplantation at our institute over the last 22 years. In our series of transplants performed before the year 2000, one IAK patients maintained a functional islet graft for >1 year, despite baseline PRA+. Two IAK recipients who developed PRA+ with DSA while on immunosuppression maintained islet allograft function for >13 years (*28*, *30*). Six SIK recipients (1994–96 trial) maintained excellent kidney graft function for >600 days without episodes of rejection, despite loss of islet graft function.

After 2000, three I-BMT recipients appeared PRA+ at the retesting of baseline samples by ELISA and Flow. All remained PRA+ during follow-up while under immunosuppression: two of them lost graft function within 5 months, while one showed sustained function while under immunosuppression. A recent report demonstrated a negative association of pretransplant PRA+ and the outcome of ITA (*16*). Further studies on larger cohorts of patients will allow for the conclusive assessment of the impact of basal PRA+ on the fate of islet allografts.

The development of PRA+ after islet transplantation has been associated with impairment of graft function measured as reduction of C-peptide during metabolic testing (*13*) and overall metabolic control assessed by the Ryan β-score (*17*). Loss of islet graft function has also been reported after allosensitization in islet transplant recipients (*14*, *20*). In our study, most patients remained PRA-negative while on immunosuppression (*7*), with 11 cases (7 before and 4 after the year 2000) displaying PRA+ by CDC in association with lowering of immunosuppression trough levels and/or infection episodes.

Development of DSA associated with loss of allogeneic islet function has been reported in experimental transplantation (*39*) and in a recent clinical case (*18*). In our series, DSA+ was detected in selected patients both before and after islet transplantation, although no definitive association with graft outcome could be determined from our analysis.

The lack of PRA+ in our recent trials in patients under adequate immunosuppressive management indicates that the therapeutic protocols utilized may have effectively prevented alloantibody production. Notably, most of the trials were based on high trough levels of sirolimus and low doses of tacrolimus (*7*, *30*), which might have contributed to this observation. Furthermore, our data suggests that monitoring of PRA may not have great value if therapeutic levels of immunosuppressive drugs are achieved, as reported earlier (*15*). The use of antidonor mixed lymphocyte reaction in vitro has been recently proposed as a valuable tool for the immune monitoring of islet allograft recipients as it could be associated to graft dysfunction in islet allograft recipients (*15*, *40*, *41*).

Retesting of archived sera by CDC, ELISA, and Flow demonstrated that a number of samples resulting negative or borderline by CDC were frankly positive by the other methods. It has been suggested that CDC may be less sensitive for the detection of allosensitization and that ELISA and Flow maybe more suitable and reproducible to perform (*42*). Our old trials relied on PRA by CDC, and this may have contributed to misdiagnose previous allosensitization in some cases. Indeed, we have observed that selected patients who displayed PRA+ at baseline when retesting was performed with the new technology maintained positive values during the follow-up under immunosuppression, which may have contributed, at least in some cases, to the poor islet graft outcome recorded.

The most striking data emerging from the present study is the invariable development of allosensitization in virtually all patients who discontinued immunosuppression. This

was associated with the development of DSA+ in 75% (12 of 16) of the patients studied after the year 2000 who had received either ITA or I-BMT. Our results confirm and extend previous observations of allosensitization after discontinuation of immunosuppression in a smaller number of cases (*8*, *19*, *21*, *43*, *44*).

Improved metabolic control, quality of life, and prevention of severe hypoglycemia can be consistently obtained after islet transplantation (*3*, *4*, *7*), even with partial graft function requiring exogenous insulin to maintain A1c in the target range. The risk of allosensitization after transplantation of allogeneic tissues is a common problem of solid organ and cellular grafts. In the case of pancreatic islets, the need for relatively large masses of insulin-producing cells to achieve insulin independence has led to the use of multiple donors (sequential or pooled islet preparations) per recipient (*5*, *7*, *45*, *46*). This may increase the risk for allosensitization to multiple HLAs and represents a major concern in the case the patient discontinues immunosuppression after transplantation. Notably, allosensitization may consist in the development of DSA and also of alloantibodies to additional nondonor-specific antigens. The clinical significance of the presence of these alloantibodies per se needs further evaluation also in view of recently reports showing that the strength and/or titer (*47*) of the antibodies and their ability to fix complement (*48*) have higher correlation with graft dysfunction in solid organ transplantation. Although the relevance of this phenomenon on the outcome of subsequent allografts has not been established yet, there is a concern for potentially limiting future therapeutic options (namely, subsequent islet, pancreas and/or kidney transplantation for end-stage kidney disease) in recipients who discontinued immunosuppression after islet graft loss. It is noteworthy that the majority of subjects with long-standing (>15 years) T1DM who received ITA in recent clinical trials have been highly selected for minimal kidney dysfunction (*7*); therefore, the slow progression of diabetic nephropathy in these patients seems to indicate that it is unlikely that they will develop ESRD that would require kidney transplantation in the future.

Development of approaches allowing for the achievement of insulin independence from a single donor islet preparation per recipient (*25*, *43*, *49–51*) will be of assistance in reducing the risk for multiple donor allosensitization in the future. Also, implementation of more stringent donor/recipient HLA matching could contribute reducing the risk of allosensitization. In light of the observed lack of alloantibody production in patients while being on immunosuppression, it is conceivable that development of safe immunosuppressive protocols (i.e., weaning protocols over extended periods of time) after islet graft failure could contribute reducing the risk of sensitization in these patients.

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