Long-Term Metabolic and Immunological Follow-Up of Nonimmunosuppressed Patients With Type 1 Diabetes Treated With Microencapsulated Islet Allografts

Four cases

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OBJECTIVE—To assess long-term metabolic and immunological follow-up of microencapsulated human islet allografts in nonimmunosuppressed patients with type 1 diabetes (T1DM).

RESEARCH DESIGN AND METHODS—Four nonimmunosuppressed patients, with long-standing T1DM, received intraperitoneal transplant (TX) of microencapsulated human islets. Anti-major histocompatibility complex (MHC) class I–II, GAD65, and islet cell antibodies were measured before and long term after TX.

RESULTS—All patients turned positive for serum C-peptide response, both in basal and after stimulation, throughout 3 years of posttransplant follow-up. Daily mean blood glucose, as well as HbA_{1c} levels, significantly improved after TX, with daily exogenous insulin consumption declining in all cases and being discontinued, just transiently, only in patient 4. Anti-MHC class I–II and GAD65 antibodies all tested negative at 3 years after TX.

CONCLUSIONS—The grafts did not elicit any immune response, even in the cases where more than one preparation was transplanted, as a unique finding, compatible with encapsulation-driven "bioinvisibility" of the grafted islets. This result had never been achieved with the recipient's general immunosuppression.

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The primary goal of this study, following our previous report (1), was to determine long-term safety of encapsulated human islet (HI) transplant (TX), upon completion of two additional cases. The following parameters were examined: *1*) TX-related adverse reactions; *2*) TX-directed immune destruction in nonimmunosuppressed recipients; and *3*) sensitization to grafted encapsulated islet cell antigens. We also examined *1*) changes in exogenous insulin consumption; *2*) levels of prior negative serum C-peptide response; *3*) changes in severe nocturnal hypoglycemia, defined by blood glucose (BG) <40 mg/dL (patients 1 and 2) (2); and *4*) changes in HbA_{1c} plasma levels.

RESEARCH DESIGN AND METHODS

Human islet procurement

HIs were isolated from single donor pancreases, according to the Edmonton protocol (3). Islet preparations from our laboratory were grafted in patients 1 and 2. We also used HIs procured at the University of Illinois at Chicago (UIC) (patients 3 and 4). The "UIC HIs" were isolated using a modified Ricordi's method and passed the product release criteria including viability, purity, and endotoxin levels <5 endotoxin units/g (EU/g), as required by the U.S. Food and Drug Administration. The UIC HIs could be used in our Center because there was no suitable U.S. recipient available for such a given islet preparation. This scenario happened because the HI yield was insufficient to achieve the required 5,000 islet equivalents (IEQ)/kg body wt of listed U.S. recipients. Islet morphology, viability, and functionality assessments were performed before and after microencapsulation, showing *1*) purity >80%; *2*) viability >90%; and *3*) stimulation index upon static incubation with glucose >5 above baseline.

Islet microencapsulation

The selected islet batches were encapsulated in ultra-purified, endotoxin-free sodium alginate prepared in-house (patent number WO 2009/093184 A1) by our method (4).

Patient selection

Four patients with long-standing type 1 diabetes (T1DM) were selected, as previously reported (1).

Clinical, metabolic, and immunological evaluation

All clinical and metabolic parameters were carefully acquired before and strictly monitored after TX.

Basal pre-TX clinical assessment. Complete blood chemistry, including all metabolic parameters (HbA_{1c}; daily glucose profiles after and 3 consecutive months before entering the trial), was performed.

Post-TX assessment. All grafted patients underwent hourly BG and exogenous insulin supplement monitoring to keep

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Table 1—*Summary of clinical, metabolic, and immunological data of the transplanted patients throughout long-term follow-up (patients 1 and 4 received more than one graft)*

	Patient 2	Patient 3	Patient 1	Patient 4
Duration of T1DM (years)	20	21	25	27
Pre-TX severe hypoglycemia (events/week)	1	3	4	4
Post-TX severe hypoglycemia (events/week)	0	0	0	0
Body weight at the time of transplant (kg)	78	68	70	68
			72*	66†‡
Mass of islets implanted (IEQ)	650,000	540,000	400,000	500,000
			400,000*	500,000†
				600,000‡
Total islet mass (IEQ)	NA	NA	800,000	1,600,000
Pre-TX mean BG (mg/dL)	235 ± 78	180 ± 63	275 ± 98	247 ± 55
Post-TX mean BG (mg/dL)				
6 months	155 ± 44	103 ± 34	115 ± 56*	145 ± 36†‡
12 months	174 ± 54	176 ± 50	167 ± 58	151 ± 18
15 months	190 ± 18	170 ± 63	175 ± 24	176 ± 12
18 months	165 ± 44	123 ± 14	180 ± 36	170 ± 38
24 months	176 ± 31	162 ± 15	198 ± 16	176 ± 26
30 months	195 ± 06	Dropout	241 ± 20	177 ± 24
36 months	201 ± 41		208 ± 16	204 ± 16
Pre-TX sCPR (ng/mL)	Undetectable	Undetectable	Undetectable	Undetectable
Post-TX sCPR (ng/mL)				
3 months	Premeal = 0.25, postmeal = 1.00	Premeal = 0.63, postmeal = 1.30	Premeal = 0.20, postmeal = 0.90	Premeal = 0.57, postmeal = 1.10†
6 months	Premeal = 0.41, postmeal = 0.85	Premeal = 0.58, postmeal = 0.91	Premeal = 0.10, postmeal = 0.42*	Premeal = 0.33, postmeal = 0.81†‡
12 months	Premeal = 0.35, postmeal = 0.80	Premeal = 0.40, postmeal = 0.75	Premeal = 0.25, postmeal = 0.50	Premeal = 0.35, postmeal = 0.73
18 months	Premeal = 0.30, postmeal = 0.75	Premeal = 0.20, postmeal = 0.50	Premeal = 0.18, postmeal = 0.48	Premeal = 0.31, postmeal = 0.74
24 months	Premeal = 0.18, postmeal = 0.61	Premeal = 0.10, postmeal = 0.44	Premeal = 0.26, postmeal = 0.58	Premeal = 0.35, postmeal = 0.70
30 months	Premeal = 0.15, postmeal = 0.47	Dropout	Premeal = 0.15, postmeal = 0.45	Premeal = 0.46, postmeal = 0.74
36 months	Premeal = 0.10, postmeal = 0.51		Premeal = 0.26, postmeal = 0.55	Premeal = 0.34, postmeal = 0.76
Daily exogenous insulin (IU) pre-TX	37	36	32	32
Daily exogenous insulin (IU) post-TX				
3 months	29	22	13	21†
6 months	22	20	15*	22†‡
12 months	22	18	15	20
15 months	28	20	22	20
18 months	28	16	22	20
24 months	30	18	25	26
27 months	30	Dropout	22	24
30 months	28		25	28
36 months	28		25	28
Pre-TX GHb (%)	8.7 ± 0.3	8.0 ± 0.6	9.0 ± 0.2	9.0 ± 0.4
Post-TX GHb (%)				
3 months	7.2 ± 0.4	7.2 ± 0.4	7.8 ± 0.4	8.1 ± 0.2†
6 months	7.5 ± 0.3	7.4 ± 0.2	8.2 ± 0.3*	7.2 ± 0.4†‡
12 months	7.8 ± 0.3	7.3 ± 0.3	7.2 ± 0.1	6.5 ± 0.3
15 months	7.6 ± 0.2	7.1 ± 0.5	7.6 ± 0.4	5.9 ± 0.2
18 months	8.0 ± 0.1	7.2 ± 0.1	7.4 ± 0.2	6.2 ± 0.4
24 months	7.8 ± 0.3	7.5 ± 0.3	7.7 ± 0.4	7.4 ± 0.1
27 months	7.5 ± 0.4	Dropout	7.2 ± 0.2	7.2 ± 0.2
30 months	7.3 ± 0.4		7.7 ± 0.3	6.1 ± 0.3
36 months				7.5 ± 0.1

Table 1—*Continued*

	Patient 2	Patient 3	Patient 1	Patient 4
Metabolic function				
sCPR (ng/mL), 3 months	NA		Oral glucose tolerance test BG (mg/dL)-sCPR	
0 min			150–0.075	
30 min			230–0.100	
60 min			240–0.198	
90 min			280–0.130	
120 min			300–0.209	
180 min			288–0.171	
240 min			285–0.090	
sCPR (ng/mL), 3 months	NA		Arginine	
0 min			0.26	
2 min			0.32	
4 min			0.30	
6 min			0.45	
8 min			0.31	
10 min			0.38	
sCPR (ng/mL), 3 months	NA	Glucagon test		Glucagon test†
0 min		0.047		0.71
5 min		0.017		
10 min		1.090		0.83
15 min		1.480		
30 min		0.500		0.95
40 min		0.840		0.82
60 min		0.380		0.99
120 min		0.470		0.77
sCPR (ng/mL), 12 months				Glucagon test†‡
0 min				0.18
5 min				0.59
10 min				0.56
15 min				0.62
20 min				0.74
30 min				0.71
40 min				0.85
50 min				0.90
60 min				
120 min				0.42
sCPR (ng/mL), 36 months	Dropout	Dropout		Glucagon test
0 min				0.30
5 min				0.42
10 min				0.48
15 min				0.89
20 min				0.76
30 min				0.74
40 min				0.44
50 min				0.37
60 min				
120 min				0.32
Immune monitoring (pre-TX)				
Anti-GAD 65 antibodies	Negative	Negative	Negative	Negative
Islet cell antibodies	Negative	Negative	Negative	Negative
Class I HLA	Negative	Negative	Negative	Negative
Class II HLA	Negative	Negative	Negative	Negative
Immune monitoring (3–5 years post-TX)				
Anti-GAD 65 antibodies	Negative	Negative	Negative	Negative
Islet cell antibodies	Negative	Negative	Negative	Negative
Class I HLA	Negative	Negative	Negative	Negative
Class II HLA	Negative	Negative	Negative	Negative

sCPR, serum C-peptide response. Patients 2 and 3 got a single islet graft. Patient 1 got a second graft *6 months after the first one. Patient 4 got a second graft †7 days after the first one and ‡a third graft 6 months after the first.

BG within the prefixed range (120–150 mg/dL).

Metabolic and immunological characterization. All patients, upon TX, underwent either an oral glucose tolerance test (75 g; patient 1 only) or a glucagon (1 mg i.v.) or arginine test (10 g in 250 mL saline i.v.; patient 1 only) to determine basal and poststimulation serum C-peptide response by radioimmunoassay (Myria, Milan, Italy). Islet cell antibodies, anti-GAD65 antibodies, and anti-major histocompatibility complex (MHC) class I–II antibodies were assessed before and after transplantation (Table 1) on a long-term follow-up basis. Anti-MHC class I–II antibodies were assessed by ELISA (Biotest, Waukesha, WI).

Imaging. Abdominal MRI was scheduled only if necessary, to exclude the occurrence of post-TX peritoneal lesions.

Site of transplant and intervention procedure

All patients received a transplant of microencapsulated HIs intraperitoneally, under ecography guidance and local anesthesia. The encapsulated islet suspension in saline was placed in a 60-mL syringe barrel and slowly delivered through a polypropylene catheter into the peritoneal cavity through a small incision of the abdominal wall. The injected total graft volume did not exceed 100 mL (capsules + saline). Patient 4, on his third TX, underwent abdominal laparoscopy, under general anesthesia, to visually select and optimize the TX site and possibly avoid capsule injection errors (see patient 1). In this instance, the microcapsules were evenly distributed beneath the liver and the spleen, where blood supply is high. In all TX procedures, care was taken to dispense the capsular suspension as thoroughly as possible, to prevent formation of capsules clusters. Human islet dosing varied between recipients, ranging from 5,000 to 15,000 IEQ/kg/TX (range 540,000–1,600,000 IEQ/patient). This result strictly depended on organ availability and the islet isolation rate per pancreas.

RESULTS

Clinical outcome

None of the TX recipients showed any acute, significant postoperatory side effects. BG levels were stable, both short and long term after TX. In particular, throughout 24 months of TX, daily mean BG was stable in all patients, whereas after such time, the values tended to slightly but progressively raise. Interestingly, patient 1, who had suffered for severe nocturnal hypoglycemic episodes, showed evident recovery, in conjunction with stabilization of BG profiles. The natural history of this pilot study is summarized in Table 1, throughout 3 consecutive years of post-TX follow-up. At this time, the study was terminated and the patients were seen once a year. So far, at 7 years post-TX, all patients are in good health and are fully back to their original exogenous insulin therapy regimens.

Immunological findings

As a unique finding, no anti-MHC class I–II or anti-GAD65 antibodies or islet cell antibodies were detected in any of the transplanted patients throughout 5 years of post-TX follow-up (Table 1).

Microcapsule retrieval

Patient 1, 5 years after TX, back to his original insulin schedule, started complaining of abdominal discomfort. In our Center, upon palpation of the abdomen, we found a small mass that ultrasound scan identified as a hyperechoic cyst-like formation. The cyst, situated in the rectus anterior muscle, was surgically removed and consisted of a 3- to 4-cm fibrotic lump that contained mostly intact capsules with no more viable islet cells inside. Obviously, the original capsules, in this instance, had been mistakenly injected beneath the muscle fascia rather than intraperitoneally, thus resulting in the cyst formation.

CONCLUSIONS—Our alginate/polyaminoacidic encapsulation system has been confirmed to represent a powerful tool for immunoprotection of HI grafts (5), as proven by the absence of a wide array of islet cell-directed as well as anti-MHC class I–II antibodies (6). Hence, microcapsules provided the islet grafts with bioinvisibility, according to U.S. Food and Drug Administration criteria. In our opinion, this was the most important finding of the study. Obviously, the partial and transient metabolic benefits obtained by the treatment reflect limitations of this microcapsules generation, with special regard to their size in relation to TX site. Moreover, HIs can be moved through long distances with no loss of their viability and function (7). We maintain that smaller-size microcapsules could permit access to TX sites possibly associated with better functional exchange, thereby complying, more efficiently, with metabolic requirements of patients with T1DM.

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