Graves Hyperthyroidism After Stopping Immunosuppressive Therapy in Type 1 Diabetic Islet Cell Recipients With Pretransplant TPO Autoantibodies

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OBJECTIVE — After an initially successful islet cell transplantation, a number of patients return to C-peptide negativity, and therefore immunosuppressive therapy is discontinued. Some are then found to have developed Graves disease. We examined the risk of Graves disease after immunosuppression.

RESEARCH DESIGN AND METHODS — Immunosuppressive therapy was stopped in 13 type 1 diabetic islet cell recipients who had received one course of antithymocyte globulin and maintenance doses of mycophenolate mofetil and a calcineurin inhibitor. None had a history of thyroid disease.

RESULTS — In four patients, clinical Graves hyperthyroidism was observed within 21 months after discontinuation and 30–71 months after the start of immunosuppressive therapy. All four patients exhibited a pretransplant positivity for thyroid peroxidase (TPO) autoantibodies, while the nine others were TPO negative pre- and posttransplantation.

CONCLUSIONS — Type 1 diabetic recipients of islet cell grafts with pretransplant TPO autoantibody positivity exhibit a high risk for developing Graves hyperthyroidism after immunosuppressive therapy is discontinued for a failing graft.

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Islet cell transplantation has been shown to reproducibly achieve metabolic correction in nonuremic type 1 diabetic patients (1,2). However, in the years following transplantation, several of them return to C-peptide negativity and thus to a discontinuation of their immunosuppressive therapy (2).

RESEARCH DESIGN AND METHODS

— Between 1999 and 2002, 17 type 1 diabetic patients (median age 43 years [range 25–56]) received an islet cell graft under one course of antithymocyte globulin (ATG-Fresenius) and maintenance therapy with mycophenolate mofetil (MMF) plus cyclosporine ($n = 9$) or tacrolimus ($n = 8$). In 13 of the patients, immunosuppressive therapy was stopped (calcineurin inhibitor first) 6–66 months later because plasma C-peptide levels had dropped under 0.2 ng/dl. They were further monitored for side effects from the intervention protocol.

In terms of autoimmune status, HLA-DQA1-DQB1 and DR3 genotypes and single nucleotide polymorphisms were determined to be susceptibility markers (3, rev. in 4), lymphocytes were phenotyped (5), and autoantibodies (islet cell antibody, insulin antibody, GAD antibody, insulinoma antigen 2 antibody) were measured (6).

Data are presented as median (range). For comparison of patient subgroups, the Mann-Whitney *U* test was used for quantitative variables and the Fisher's exact test was used for binary variables. Differences were considered significant for $P < 0.05$.

RESULTS

— Clinical Graves disease was diagnosed in 4 of 13 subjects (31%) at 2–21 months after withdrawal of immunosuppressants and 30–71 months after transplantation. Diagnosis was confirmed by suppressed thyrotropin (TSH) levels (<0.01 mIU/l), elevated free thyroxin (20.4–67.7 ng/l; normal 9.3–17.0) and free 3,5,3′-triiodothyronine (6.3–16.9 ng/l; normal 2.6–4.4) levels, and positivity for thyrotropin receptor (TSHR) autoantibodies (3.2–23.8 units/l; normal <1). All the patients exhibited a diffusely increased thyroid technetium-99 uptake (5–17%; normal 1–5).

No differences in pretransplant characteristics were noticed among the four Graves-positive and the nine Graves-negative patients except that all the Graves-positive patients and none of the others were positive for thyroid peroxidase (TPO) autoantibodies ($P = 0.001$) (Table 1). The Graves-positive patients also tended to be more polymorphic in the protein tyrosine phosphatase nonreceptor type 22 (*PTPN22*) susceptibility gene (three of four vs. one of nine patients, $P = 0.051$). There were no differences in age, sex, smoking habits, TSH before transplantation, iodide deficiency status, duration of diabetes, and presence of diabetes-related autoantibodies (data not shown).

The respective doses of immunosuppressants were similar among the Graves-positive and Graves-negative patients: ATG-Fresenius (cumulative median 24.5

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Table 1—*Course of thyroid autoantibody positivity in recipients of islet cell grafts developing Graves hyperthyroidism following discontinuation of immunosuppressive therapy*

	Pretransplantation (none)		Posttransplantation					
			CI and MMF		MMF continued after stopping CI		None after stopping CI and MMF	
	TPO-Ab	TSHR-Ab	TPO-Ab	TSHR-Ab	TPO-Ab	TSHR-Ab	TPO-Ab	TSHR-Ab
Graves (n = 4)								
M.R.	+	−	−	−	+ (2)	+ (2)	+	+
S.V.	+	−	−	−	−	−	+ (14)	+ (11)
V.G.J.	+	−	−	−	+ (3)	−	+	+ (8)
R.I.	+	−	−	−	−	−	+ (8.5)	+ (8.5)
No Graves (n = 9)	−	−	−	−	−	−	−	−

The number in parentheses indicates the month at which thyroid antibody positivity was first detected after stopping the calcineurin inhibitor. Ab, antibody; CI, calcineurin inhibitor.

mg/kg [range 24.0–27.0] vs. 24.3 mg/kg [22.0–30.0], $P = 0.64$), trough levels of tacrolimus (median 4.5 ng/dl [4.0–6.5] vs. 6.0 ng/dl [4.1–6.6], $P = 0.54$) and cyclosporine (133 μg/l [114–153] vs. 143 μg/l [112–165], $P = 0.69$), and daily MMF doses (2.0 g/day [1.0–2.0] vs. 2.0 g/day [1.5–2.0], $P = 1.00$).

T-cell counts were similar before transplantation but tended to be lower in the pre-Graves patients during immunosuppressive therapy; this was particularly reflected in the CD4+ subset counts at 3 months posttransplantation (PT) (93 mm^3 [60–167] vs. 154 mm^3 [43–417], $P = 0.06$) and at 9 months PT (152 mm^3 [98–196] vs. 285 mm^3 [134–516], $P = 0.06$).

During immunosuppressive therapy, the four TPO autoantibody–positive patients became TPO autoantibody negative and remained so (Table 1). When it was discontinued, TPO autoantibodies reappeared in all four patients with detection at 2 and 14 months after stopping the calcineurin inhibitor (Table 1). In addition, TSHR autoantibodies also appeared in these patients between 2 and 11 months after stopping the calcineurin inhibitor. Of the nine patients that were TPO autoantibody negative before transplantation, none became positive for TPO or TSHR autoantibodies during a 28- to 85-month follow-up period after discontinuation of the immunosuppressants. All Graves-positive patients also exhibited increases in one or more diabetes-related autoantibodies after drug withdrawal, but this was also the case in six of nine Graves-negative patients ($P = 0.49$).

CONCLUSIONS — We report the development of Graves hyperthyroidism in four type 1 diabetic patients in whom immunosuppressive therapy had been stopped 2–21 months earlier for a failing islet cell graft. These four patients exhibited a pretransplant positivity for TPO autoantibodies without clinical or biochemical signs of thyroid disease. Among the nine recipients who were negative for TPO autoantibodies pretransplantation, none developed Graves hyperthyroidism. TPO seropositivity has been associated with an increased risk for autoimmune hypothyroidism (7) and is present in 60–70% of patients with Graves hyperthyroidism. In type 1 diabetic patients, TPO autoantibodies were found in 30% of patients, but Graves hyperthyroidism was found in only 1–2% (8,9). We now observe that the presence of TPO autoantibodies in type 1 diabetic patients might be predictive for their susceptibility of developing Graves hyperthyroidism after transient immunosuppressive therapy, at least for an islet cell transplant protocol.

Immunosuppressive therapy decreased TPO autoantibody titers under detection levels. Its discontinuation resulted within 21 months with the reappearance of TPO autoantibodies, as well as an appearance of TSHR autoantibodies and clinical signs of hyperthyroidism. We hypothesize that the drug-induced lymphopenia in patients with previously existing occult autoimmune thyroiditis predisposes to a reactivation of autoimmune mechanisms when T-cells are repopulated and thus to an aberrant immune reconstitution. Depletion of T-cells has been found previously to be associated with a higher risk for Graves disease (10) in patients receiving ATG for aplastic anemia (11) or anti-CD52 monoclonal antibody for multiple sclerosis (12). The profound decrease in CD4+ lymphocytes in HIV patients is also considered to predispose for autoimmune syndromes when the T-cell compartment is reconstituted after antiretroviral therapy (13).

In our patients, the ATG-induced lymphopenia and subsequent repopulation of T-cells may have altered the immune state leading to an autoreactivity against thyrocyte antigens such as TSHR. In this respect, the higher frequency of polymorphisms in the *PTPN22* susceptibility gene is of interest because it may predispose to the formation of autoreactive lymphocytes (14). The administration of the calcineurin inhibitor and of MMF probably suppressed the autoreactivity induced by ATG and its lymphocyte depletion. This is in line with data showing that calcineurin inhibitors efficiently inhibit functions of CD4+ memory phenotype cells (15). Future studies should examine whether slower tapering of the immunosuppressive agents can avoid development of pathogenic autoimmune activities in TPO autoantibody–positive recipients. The use of lower cumulative doses of ATG should also be considered in TPO autoantibody–positive patients, as they would lead to a less pronounced and shorter T-cell depletion.

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