Graves’ Hyperthyroidism after Stopping Immune Suppressive Therapy in Type 1 Diabetic Islet Cell Recipients with Pretransplant TPO-Autoantibodies

Running title: Graves’ Hyperthyroidism after Islet Transplantation

Pieter Gillard M.D., Ph.D. ¹,², Volkert Huurman, M.D., Ph.D. ³, Bart Van der Auwera Ph.D. ¹, Brigitte Decallonne M.D., Ph.D. ², Kris Poppe M.D., Ph.D. ¹, Bart O. Roep Ph.D. ³, Frans Gorus M.D., Ph.D. ¹, Chantal Mathieu M.D. Ph.D.², Daniel Pipeleers M.D. Ph.D. ¹, Bart Keymeulen M.D. Ph.D. ¹

1. Diabetes Research Center and University Hospital Brussels, Brussels Free University-VUB, Laarbeeklaan 103, B-1090 Brussels, Belgium
2. Department of Endocrinology, University Hospital Gasthuisberg, KULeuven, Herestraat 49, B-3000 Leuven, Belgium
3. Department of Immunohematology and Blood Transfusion and Department of Surgery, Leiden University Medical Center, Albinusdreef 2, NL-2300 Leiden, The Netherlands
1.2. JDRF Center for Beta Cell Therapy in Diabetes, Central Unit Medical Campus-VUB, Brussels 1090, Belgium

Address for Correspondence:
Bart Keymeulen
Email: Bart.Keymeulen@vub.ac.be

Submitted 31 December 2008 and accepted 14 June 2009.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
**Objective:** After an initially successful islet cell transplantation, a number of patients return to C-peptide-negativity and are therefore discontinued in immune suppressive therapy. Some were found to develop Graves’ disease.

**Research Design and Methods:** Immune suppressive 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 4 patients clinical Graves' hyperthyroidism was observed within 21 months after discontinuation, and 30 to 71 months after start of immune suppressive therapy. All four 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 immune suppressive therapy is discontinued for a failing graft.
Islet cell transplantation has been shown to reproducibly achieve metabolic correction in non-uremic type 1 diabetic patients (1;2). However, during the years following transplantation several of them return to C-peptide-negativity and thus to a discontinuation of their immune suppressive therapy (2).

**RESEARCH DESIGN AND METHODS**

Between 1999 and 2002, 17 type 1 diabetic patients (age 43 yrs, 25 to 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 them, immune suppressive therapy was stopped (calcineurin inhibitor first) 6 to 66 months later because plasma C-peptide levels had dropped under 0.2 ng/dl. They were further monitored for side effects of the intervention protocol.

In terms of autoimmune status, HLA-DQA1-DQB1 and DR3 genotypes and single nucleotide polymorphisms were determined as susceptibility markers (3, reviewed in 4), lymphocytes were phenotyped (5) and autoantibodies (islet cell antibody-ICA, insulin antibody-IA2-A, glutamic acid decarboxylase antibody-GADA, insulinoma antigen 2 antibody-IA2-A) 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 for binary variables. Differences were considered significant for P<0.05.

**RESULTS**

Clinical Graves’ disease was diagnosed in four out of thirteen subjects (31%) at month 2 to 21 following withdrawal of immunosuppressants, and 30 to 71 months after transplantation. Diagnosis was confirmed by suppressed thyrotropin (TSH)-levels (<0.01 mIU/l), elevated free thyroxin (20.4 to 67.7 ng/l; normal 9.3-17.0) and free 3,5,3’-triiodothyronine (6.3 to 16.9 ng/l; normal 2.6-4.4) levels and positivity for thyrotropin receptor (TSHR)-autoantibodies (3.2 to 23.8 U/l; normal <1). They all exhibited a diffusely increased thyroid technetium-99 uptake (5 to 17%; normal 1-5).

No differences in pretransplant characteristics were noticed between the 4 Graves’-positive and 9 Graves’-negative patients except that for a TPO-autoantibody-positivity in all Graves’-positive patients and in none of the others (Table 1; P=0.001). Graves’-positive patients also tended to be more polymorphic in the protein tyrosine phosphatase non-receptor type 22 (PTPN22)-susceptibility gene (3/4 versus 1/9; P=0.051). There were no differences in age, gender, 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 between the Graves’-positive and Graves’-negative patients: ATG-Fresenius® (cumulative 24.5 mg/kg, 24.0-27.0 versus 24.3 mg/kg, 22.0-30.0, P=0.64), trough levels of tacrolimus (mean 4.5 ng/dl, 4.0-6.5 versus 6.0 ng/dl, 4.1-6.6, P=0.54) and cyclosporine (133 µg/l, 114-153 µg/l versus 143 µg/l, 112-165; P=0.69) and daily MMF dose (2.0 g/d, 1.0-2.0 versus 2.0 g/d, 1.5-2.0; P=1.00).

T-lymphocyte counts were similar before transplantation but tended to be lower in the pre-Graves’ patients during immune suppressive therapy; this was particularly reflected in the CD4 + subset counts at 3 months PT (93/mm³, 60-167 versus 154/mm³, 43-417; P=0.06) and at 9 months PT (152/mm³, 98-196 versus 285/mm³, 134-516; P=0.06).

During immune suppressive 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, with detection at months 2 and 14 after stopping the calcineurin-inhibitor (Table 1). In addition, TSHR-autoantibodies also appeared in these patients between months 2 and 11 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 following stop of the immunosuppressants. All Graves’-positive patients also exhibited an increase in one or more diabetes related autoantibodies after drug withdrawal but this was also the case in 6 out of 9 Graves’-negative patients (P=0.49).

CONCLUSIONS

We report the development of Graves’ hyperthyroidism in four type 1 diabetic patients in whom immune suppressive therapy had been stopped 2 to 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 did develop Graves’ hyperthyroidism. TPO-seropositivity has been associated with an increased risk for autoimmune hypothyroidism (7) and is present in 60 to 70% of patients with Graves’ hyperthyroidism. In type 1 diabetes, TPO-autoantibodies were found in 30% of patients, but Graves’ hyperthyroidism only in 1 to 2 percent (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 following transient immune suppressive therapy, at least for an islet cell transplant protocol.

Immune suppressive therapy decreased TPO-autoantibody titers under detection levels. Its discontinuation resulted within 21 months in reappearing TPO-autoantibodies, as well as in 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-lymphocytes are repopulated and thus to an aberrant immune reconstitution. Depletion in T-lymphocytes has been previously 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-lymphocytes compartment is reconstituted following anti-retroviral therapy (13).

In our patients, the ATG induced lymphopenia and subsequent T-lymphocytes repopulation may have altered the immune state leading to an autoreactivity against thyrocyte antigens such as the thyrotropin receptor. In this respect, the higher frequency of polymorphisms in the PTPN22-susceptibility gene is of interest since it may predispose to the formation of autoreactive lymphocytes (14). The administration of the calcineurin inhibitor and of mycophenolate mofetil 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 immune suppressive 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-lymphocyte depletion.

ACKNOWLEDGEMENTS

The authors acknowledge the staff of the Beta Cell Bank, the Belgian Diabetes Registry, the Diabetes Research Center-VUB and the Clinical Biology Department of UZ Brussel. This study was supported by a Center Grant from the Juvenile Diabetes Research Foundation (JDRF, 4-2001-434), by the Belgium Program on Interuniversity Poles of Attraction initiated by the Belgian State (IAP P5/17) and by the Fund for Scientific Research-Flanders (FWO). Drs. B. Keymeulen, Brigitte Decallonne and C. Mathieu are Senior Clinical Investigators of FWO.

Disclosure: No conflict of interest
Graves’ Hyperthyroidism after Islet Transplantation

REFERENCES
15. Pearl JP, Parris J, Hale DA, Hoffmann SC, Bernstein WB, McCoy KL, Swanson SJ,
Mannon RB, Roederer M, Kirk AD: Immunocompetent T-cells with a memory-like phenotype are the dominant cell type following antibody-mediated T-cell depletion. *Am J Transplant* 5:465-74, 2005
Table 1. Course of thyroid autoantibody positivity in recipients of islet cell grafts developing Graves’ hyperthyroidism following stop of immune suppressive therapy

| Patient | Pretransplantation | Posttransplantation | | | | | |
|---------|------------------|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|         | IS Therapy | CI + MMF | MMF continued | None | CI and MMF | | | |
|         | TPO-Ab | TSHR-Ab | TPO-Ab | TSHR-Ab | TPO-Ab | TSHR-Ab | TPO-Ab | TSHR-Ab | TPO-Ab | TSHR-Ab |
| Graves (n=4) | | | | | | | | | | |
| MR      | None | CI + MMF | MMF continued | None | CI and MMF | | | |
|         | TPO-Ab | TSHR-Ab | TPO-Ab | TSHR-Ab | TPO-Ab | TSHR-Ab | TPO-Ab | TSHR-Ab | TPO-Ab | TSHR-Ab |
| SV      | + | - | - | - | + (2) | + (2) | + | + | + (14) | + (11) |
| VGJ     | + | - | - | - | + (3) | - | + | + | + (8) | |
| RI      | + | - | - | - | - | - | + (8.5) | + (8.5) | |
| No Graves (n=9) | - | - | - | - | - | - | - | - | - | - |

Mycophenolate Mofetil (MMF); Calcineurin inhibitor (CI); Thyroid peroxidase autoantibodies(TPO-Ab); Thyrotropin receptor autoantibodies (TSHR-Ab); Immune suppression (IS). The (number) indicates the month at which thyroid antibody positivity was first detected after stopping the calcineurin-inhibitor (CI)