Allogeneic islet transplantation with monitoring of islet-specific cellular autoimmunity in a Japanese patient with type 1 diabetes: A case report

Daisuke Chujo1,2,3, Toshiaki Kurokawa4, Akitsu Kawabe1, Nobuyuki Takahashi2, Fuyuki Inagaki5, Koya Shinohara1, Shotaro Hagiwara6, Yoshihiro Edamoto7, Norio Ohmagari8, Fumihiko Hinoshita9, Tsuyoshi Tajima10, Hiroshi Kajo2, Hiroshi Ohtsu11, Nobuyuki Takemura5, Shinichi Matsumoto1, Masayuki Shimoda1*

1Pancreatic Islet Transplantation Project, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan, 2Department of Diabetes, Endocrinology and Metabolism, National Center for Global health and Medicine, Tokyo, Japan, 3Center for Clinical Research, Toyama University Hospital, Toyama, Japan, 4Department of Surgery, JCHO Tokyo Takanawa Hospital, Tokyo, Japan, 5Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, National Center for Global Health and Medicine, Tokyo, Japan, 6Faculty of Medicine, University of Tsukuba, Ibaraki, Japan, 7Department of Surgery, Secomedic Hospital, Chiba, Japan, 8Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan, 9Nephrology, National Center for Global Health and Medicine, Tokyo, Japan, 10Radiology, National Center for Global Health and Medicine, Tokyo, Japan, and 11Center for Clinical Sciences, National Center for Global Health and Medicine, Tokyo, Japan

Keywords
Autoimmune response, Type 1 diabetes mellitus, Islet transplantation

*Correspondence
Masayuki Shimoda
Tel: +81-3-3202-7181
Fax: +81-3-5273-6885
E-mail address: mshimoda@hosp.ncgm.go.jp

J Diabetes Investig 2022; 13: 741–745
doi: 10.1111/jdi.13715

Clinical Trial Registry
The islet transplantation was performed based on the clinical trial ‘Clinical Study of Allogeneic Islet Transplantation for Treatment of Type 1 Diabetes’ (UMIN000014381).

INTRODUCTION
Islet transplantation is an effective treatment for unstable type 1 diabetes1. However, the lack of a valid method to detect rejection or the occurrence of autoimmunity after transplantation is a challenge. Currently, the only way to identify the cause of post-transplant recurrent diabetes is histological analysis of the islets by liver biopsy. However, biopsies are rarely carried out due to their unreliability and invasive nature.

Although it has high potential, as shown in the first successful case of living islet transplantation2, until recently, islet transplantation has only been examined in a small-scale clinical study in Japan3.

Here, we report a case of allogeneic islet transplantation to a type 1 diabetes patient in Japan. As a noteworthy evaluation, we measured the reactivity of the recipient’s cytotoxic T cells to multiple islet-related autoantigens over time before and after

Received 1 September 2021; revised 1 November 2021; accepted 16 November 2021
## Table 1 | Islet function and laboratory values

|                      | Pre  | Day 28 | Day 75 | Day 90 | Day 120 | Day 150 | Day 180 | Day 270 | Day 365 | Day 545 | Day 730 |
|----------------------|------|--------|--------|--------|---------|---------|---------|---------|---------|---------|---------|
| Bodyweight (kg)      | 69.0 | 66.8   | 66.8   | 67.2   | 67.4    | 67.8    | 69.1    | 68.6    | 71.2    | 70.3    |         |
| BMI (kg/m²)          | 22.5 | 21.0   | 21.8   | 21.9   | 22.0    | 22.2    | 22.1    | 22.6    | 22.4    | 23.2    | 23.0    |
| HbA1c (%)            | 8.6  | 7.2    | 6.0    | 6.2    | 7       | 7.4     | 7.2     | 7.6     | 7.5     | 7.1     | 7.0     |
| Glycated albumin (%) | 26.3 | 20.2   | 20.0   | 21.0   | 23.7    | 25.2    | 25.2    | 26.2    | 22.8    | 22.9    | 22.9    |
| FBS (mg/dL)          | 49   | 122    | 119    | 313    | 104     | 95      | 265     | 185     | 89      | 162     | 102     |
| Fasting CPR (ng/mL)  | <0.01| 0.53   | 0.3    | 0.3    | 0.11    | 0.04    | 0.14    | 0.26    | <0.01   | 0.12    | <0.01   |
| Stimulated CPR† (ng/mL) | 0.01 | 1.93   | NP     | 1.76   | NP      | 0.43    | NP      | 0.28    | 0.28    | 0.31    |
| CPR-AUC‡ (ng/mL/min) | 0.3  | 172.8  | 1272   | NP     | NP      | 31.95   | NP      | 14.4    | 22.2    | 16.35   |
| Proinsulin (pmol/L)  | <3.1 | <3.1   | 12.2   | 3.8    | <3.1    | <3.1    | <3.1    | NP      | NP      | <3.1    |
| Urate CPR (µg/day)   | <0.1 | 10.5   | 0.3    | 1.29   | NP      | NP      | NP      | 3.4     | NP      | 0.6     |
| Insulin dose (U/day) | 54   | 50     | 43     | 41     | 45      | 45      | 46      | 51      | 53      | 59      |
| SUITO index          | –1.07| 11.94  | 8.04   | 7.38   | 4.02    | 1.88    | 2.76    | 3.2     | 0.58    | 1.82    | 0.38    |
| β-Score              | 2    | 3      | 5      | NP     | NP      | 1       | -       | 2.2     | 0.2     | 2.0     |
| TEF score            | 0    | 4.257  | 11.47  | 11.44  | 11.29   | 9.22    | 8.26    | 3.18    | 1.2     | -4.72   | -4.71   |
| HYPO-score           | 78   | 85     | 20     | NP     | NP      | 20      | 20      | 0       | 0       | 0       |
| Clarke score         | 4    | NP     | NP     | NP     | NP      | 0       | NP      | 0       | 0       | 0       |
| MAGE (mg/dL)         | 113.25| 47.75 | 74.43  | NP     | NP      | 102.8   | 70.1    | 135     | 52.4    | 34.1    |
| Lability index (mmol/L²/h/week) | 91.91| 378.7 | 74.43  | NP     | NP      | 49.9    | 79.57   | 66      | 81.3    | 76.6    |
| Hypoglycemia unawareness | 2/month | 0    | 0      | 0      | 0       | 0       | 0       | 0       | 2       | 0       |
| Severe hypoglycemia  | 1/year| 0     | 0      | 0      | 0       | 0       | 0       | 0       | 0       | 0       |
| Anti-GAD antibody (U/mL) | 61.5 | 41.9   | 37.4   | NP     | NP      | 46.5    | NP      | 40.6    | 34.6    | 37.4    |
| Anti-IA-2 antibody (U/mL) | <0.4 | <0.4   | <0.4   | NP     | <0.4    | NP      | <0.4    | <0.4    | <0.4    | <0.4    |
| Anti-insulin antibody (U/mL) | <0.4 | <0.4   | <0.4   | NP     | <0.4    | NP      | <0.4    | <0.4    | <0.4    | <0.4    |
| Anti-HLA antibody (% panel reactive antibody) | 0 | NP | 0 | NP | NP | NP | NP | 0 | NP | 0 |
| Analysis of islet antigen-specific T-cell activity | Performed | Performed | Performed | Performed | Performed | Performed | Performed | Performed | Performed | Performed |

† Evaluated with the mixed-meal tolerance test. ‡ The area under the curve of serum C-peptide (CPR-AUC) was evaluated with the mixed-meal tolerance test using ENSURE-H (375 kcal, carbohydrate: 51.5 g, protein: 13.2 g, fat: 13.2 g; Abbott Japan, Tokyo, Japan). BMI, body mass index; CPR, C-peptide concentration; FBS, fasting blood glucose; HYPO-score, a composite hypoglycemic score calculated based on the frequency, severity, and degree of unawareness of the hypoglycemia; IEQ, islet equivalent; GAD, glutamic acid decarboxylase; HbA1c, glycated hemoglobin; HLA, human leukocyte antigens; MAGE, mean amplitude of glycemic excursion calculated by measuring the arithmetic mean of the differences between consecutive peaks and nadirs; NP, not performed; Pre, preoperatively; SUITO index, Secretory Unit of Islet Transplant Objects index (fasting serum C-peptide [ng/mL] x 1,500) / (fasting plasma glucose [mg/dL] – 63); TEF score, Transplant estimated function score calculated from the daily insulin requirement and HbA1c.
transplantation to monitor cellular autoimmunity by using the assay we established. T-cell responses are suggested as an early biomarker for the diagnosis of recurrent autoimmunity after islet transplantation.

CASE REPORT

A 48-year-old man with type 1 diabetes underwent islet transplantation from a 59-year-old female non-heart beating donor in January 2017. Islet isolation and transplantation were carried out using a previously reported method with modifications. The clinical characteristics and isolation factors are shown in Table S1.

The islets (411,230 islet equivalent [5,945 islet equivalent/kg]) were transplanted intraportally. The immunosuppressive regimen consisted of anti-thymocyte globulin and etanercept for induction. Maintenance therapy consisted of tacrolimus at trough levels of 5–10 ng/mL and mycophenolate mofetil at 1,500–2,000 mg/day. Leukopenia and renal dysfunction were not observed. At 10 months after transplantation, the patient developed Herpes zoster, but recovered with antiviral medication.

Islet graft function was evaluated for 2 years (Table 1). Glycated hemoglobin levels improved from 8.6% to 7.5% and 7.0% at 1 and 2 years after transplantation, respectively. Continuous glucose monitoring data showed improved glycemic control, in particular, hypoglycemia was reduced (Figure 1, Table S2). There was no severe hypoglycemia and very few hypoglycemia unawareness events for 2 years post-transplant (Table 1). The serum concentrations of fasting/mixed-meal stimulated C-peptide were increased from <0.01/0.01 ng/mL before transplantation to 0.53/1.93 ng/mL at 28 days after transplantation, which were maintained for up to 90 days, but began to decline thereafter. The area under the curve of serum C-peptide also increased at 28 days after transplantation, but started to decrease at 6 months (Table 1; Figure 2).

Monitoring of antibodies against class I and II human leukocyte antigens and islet autoantigens was carried out every 3–6 months (Table 1). The panel reactive anti-human leukocyte antigens antibodies remained negative, and islet-related autoantibodies neither newly developed nor increased. Proinsulin was high only at 90 and 120 days, which might indicate the destruction of the transplanted β-cells, but does not show the cause of the problem.

To monitor the emergence or recurrence of islet-specific cytotoxic cellular autoimmunity, we analyzed CD8+ T-cell responses specific for the islet antigens glutamic acid decarboxylase-65, preproinsulin, islet-specific glucose-6-phosphatase catalytic subunit-related protein and zinc transporter-8, as previously described, before and at 28 days, 90 days, 6 months, 12 months and 18 months after transplantation. The methods and peptide list for the experiments are shown in the Appendix and Table S3. The frequency of islet antigen-specific interferon-gamma-producing CD8+ T cells, indicating type 1 cytotoxic T cells, was increased at 90 days after islet transplantation, indicating the period just before the deterioration of islet graft function, and gradually decreased thereafter. Notably, the frequencies of antigens glutamic acid decarboxylase-65-, islet-specific glucose-6-phosphatase catalytic subunit-related protein- and zinc transporter-8 antibody-specific type 1 cytotoxic T cells were >1.0% of CD8+ T cells at 90 days, which were higher than during pre-transplantation (Figure 3). Taken together, our developed method might be
able to detect autoimmune flare-ups before the full-scale destruction of β-cells.

**DISCUSSION**

In current clinical practice, there is no method for detecting recurrent autoimmunity before transplanted islets are destroyed. Matsumoto *et al.*

reported allogeneic islet transplantation using potent induction immunotherapy (anti-thymocyte globulin, anakinra and etanercept). The present patient underwent islet transplantation using their immunosuppressive protocol, but without anakinra, which is not approved in Japan. Good glycemic control and endogenous insulin secretion were maintained in this patient for at least 2 years of observation. However, there was a decrease in insulin secretion after 3 months post-transplant. Islet antigen-specific T-cell activity was elevated even before the lowering of graft function became apparent.

We and others have reported the detection of islet-specific CD8 activation in cases of graft failure. However, these studies used a single antigen at a single time point. Because there are multiple immunological targets of islet destruction, we used multiple antigens and followed the patient longitudinally from before transplantation and identified the activation of islet-specific CD8 cells just before graft dysfunction. As a limitation, we do not have such immunological data from other islet transplanted patients, as this is the first case who underwent islet transplantation with the analyses in Japan. Based on our previous report showing that the frequencies of islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP)-C3 and zinc transporter-8 antibody (ZnT8)-C4 were 0.49 ± 0.78% in 15 individuals without diabetes, the frequency of the specific T cells on 90 days in the present case were considered to be elevated.

This method might have the potential to detect β-cell injury in islet transplant recipients before clinical symptoms appear. This might provide an early window for therapeutic intervention.

**ACKNOWLEDGMENTS**

This work was supported by the NCGM Intramural Research Fund (24A002). The authors thank Ms Miyuki Tsuchida and Ms Natsuko Tokuta for their coordination.

---

**Figure 3** | Longitudinal analyses of the frequencies of cytokine-producing CD8+ T cells in response to the islet antigen peptide clusters glutamic acid decarboxylase (GAD) 65-C1, preproinsulin (PPI)-C2, islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP)-C3 and zinc transporter-8 antibody (ZnT8)-C4 in the patient. An intracytoplasmic cytokine detection assay was carried out after 7-day stimulation with islet antigen peptide clusters in the presence of interleukin (IL)-2, gated to LIVE/DEAD®CD3+CD8+ T-cell populations. IFN-γ, interferon-gamma.
DISCLOSURES
Shinichi Matsumoto is a chief scientific advisor for Otsuka Pharmaceutical Factory, Inc. The other authors declare no conflict of interest.
Approval of the research protocol: The protocol for this study was approved by the Institutional Ethics Review Board and Kyoto University Specially Certified Committee for Regenerative Medicine.
Informed consent: The patient provided written informed consent for publication.
Registry and the registration no. of the study/trial: UMIN000014381.
Animal studies: N/A.

REFERENCES
1. Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med 2006; 355: 1318–1330.
2. Matsumoto S, Okitsu T, Iwanaga Y, et al. Insulin independence after living-donor distal pancreatectomy and islet allotransplantation. Lancet 2005; 365: 1642–1644.
3. Takaki T, Shimoda M. Pancreatic islet transplantation: toward definitive treatment for diabetes mellitus. Glob Health Med 2020; 2: 200–211.
4. Chujo D, Nguyen TS, Foucat E, et al. Adult-onset type 1 diabetes patients display decreased IGRP-specific Tr1 cells in blood. Clin Immunol 2015; 161: 270–277.
5. Chujo D, Kawabe A, Matsushita M, et al. Distinct phenotypes of islet antigen-specific CD4+ T cells among the 3 subtypes of type 1 diabetes. J Clin Endocrinol Metab 2020; 105: dgaa447.
6. Matsumoto S, Takita M, Chaussabel D, et al. Improving efficacy of clinical islet transplantation with iodixanol-based islet purification, thymoglobulin induction, and blockage of IL-1β and TNF-α. Cell Transplant 2011; 20: 1641–1647.
7. Maruyama K, Chujo D, Watanabe K, et al. Evaluation of cellular and humoral autoimmunity before the development of type 1 diabetes in a patient with idiopathic CD4 lymphocytopenia. J Diabetes Investig 2019; 10: 1108–1111.
8. Pinkse GG, Tysma OH, Bergen CA, et al. Autoreactive CD8 T cells associated with beta cell destruction in type 1 diabetes. Proc Natl Acad Sci USA 2005; 102: 18425–18430.
9. Chujo D, Foucat E, Takita M, et al. Emergence of a broad repertoire of GAD65-specific T-cells in type 1 diabetes patients with graft dysfunction after allogeneic islet transplantation. Cell Transplant 2012; 21: 2783–2795.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Patient’s demographic and clinical characteristics and isolation factors.
Table S2 | Parameters of continuous glucose monitoring before and after islet transplantation; Appendix: Method for the analysis of islet antigen-specific CD8+ T-cell responses.
Table S3 | Islet antigen-specific peptide clusters.