

A case of recurrent type 1 diabetes mellitus with insulinitis of transplanted pancreas in simultaneous pancreas–kidney transplantation from cardiac death donor

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Abstract

Aims/hypothesis A 41-year-old woman undergoing simultaneous pancreas–kidney transplantation from an HLA-mismatched cardiac death donor abruptly developed overt hyperglycaemia under standard immunosuppressive therapy at 48 months after transplantation. Unexpectedly, we found insulinitis in the transplanted pancreas and characterised the insulinitis.

Methods Pancreas graft biopsies were performed 3 years before and after the development of hyperglycaemia and the specimens were examined histologically.

Results Insulinitis was absent in the first biopsy, although oxidative DNA changes revealed by 8-hydroxy-2'-deoxyguanosine (8-OHdG) staining were diffusely present both in islet cells and exocrine cells. No Ki67-positive proliferating cells were seen in the islets. Anti-glutamic acid decarboxylase antibody was undetectable 6 months earlier but increased to 6.3 U/l at the development of hyperglycaemia. The level of anti-insulinoma-associated protein 2 antibody was 18.5 U/l. Insulin secretion was

severely suppressed and insulin therapy was resumed. In the second biopsy, although acute allograft rejection was minimal, insulin-positive beta cells were markedly reduced, and glucagon-positive alpha cells predominated. CD3-positive T lymphocytes, CD8-positive cytotoxic T lymphocytes and CD68-positive macrophages infiltrated around and into islets. The infiltrating cells expressed Fas ligand as well as granzyme B. More than 80% of islets were affected by insulinitis. 8-OHdG-positive cells were also present in islets and exocrine tissue. The percentage of Ki67-positive cells in total islet cells was 1.5%. There were no TUNEL-positive apoptotic cells in the islet cells. **Conclusions/interpretation** The histological features of insulinitis in transplanted pancreas were consistent with common type 1 diabetes mellitus, but the clinical course of the recurrence appeared to be more rapid.

Keywords Apoptosis · Cardiac death donor · HLA · Immunosuppression · Insulinitis · Oxidative damages · Pancreas transplantation · Replication · Rejection · Type 1 diabetes mellitus

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Abbreviations

FasL Fas ligand
IA-2 Anti-insulinoma-associated protein 2 tyrosine
phosphatase
8-OHdG 8-Hydroxy-2'-deoxyguanosine
SPK Simultaneous pancreas–kidney transplantation

Introduction

Pancreas transplantation is the only clinically established beta cell replacement therapy in patients with type 1

diabetes mellitus. However, some recipients lose graft function and resume insulin therapy after successful pancreas transplantation. The cause of graft dysfunction is mainly chronic allograft rejection, but very rarely it is recurrent type 1 diabetes mellitus [1, 2]. Organ donation from brain death patients started in Japan in 1997 but a serious shortage of brain death donors has led to the use of pancreas from marginal donors including cardiac deaths [3]. When we investigated the adequacy of using such organs for transplantation, we unexpectedly found that insulinitis was evident in one recipient of a transplant from an HLA-mismatched cardiac death donor with abrupt onset of overt hyperglycaemia under standard immunosuppressive therapy. To our knowledge, there is only one other recipient reported to have developed histologically evident insulinitis in a transplanted pancreas under HLA-mismatched and immunosuppressive conditions [4].

Materials and methods

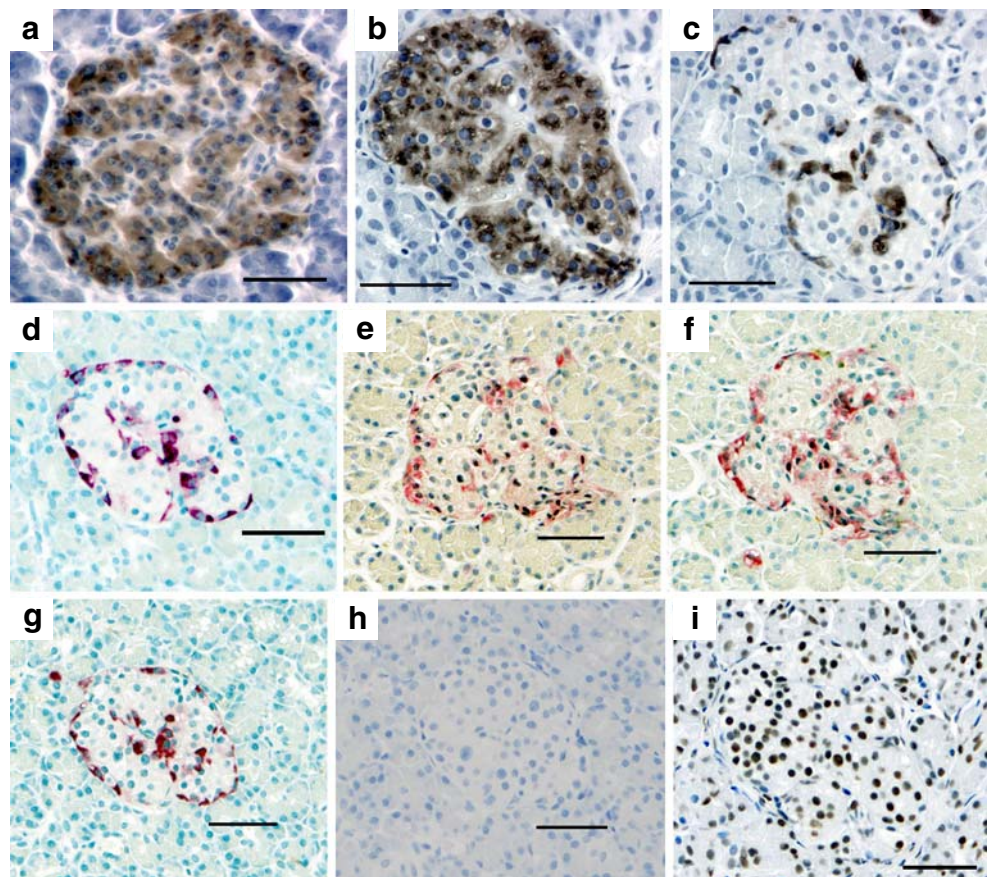
Pancreas allograft biopsy was performed by open biopsy under local anaesthesia. The patient gave written informed consent to the study. Acute allograft rejection was histologically graded according to Drachenberg's classification for pancreas graft [5] and the Banff 97 classification for kidney graft [6]. As a control, normal pancreas was obtained from the unaffected areas of surgically pancreatectomised tissue. The specimens were fixed with 10% formalin, embedded in paraffin and 5 μ m thickness specimens were stained with primary antibodies: mouse anti-insulin monoclonal antibody (Nichirei Bioscience, Tokyo, Japan), rabbit anti-glucagon polyclonal antibody (Nichirei), mouse CD3 monoclonal antibody (Nichirei), mouse CD4 monoclonal antibody (Nichirei), mouse CD8 monoclonal antibody (Nichirei), mouse CD68 macrophage monoclonal antibody (DAKO, Glostrup, Denmark), mouse monoclonal antibody Ki67 antigen (Novocastra, Newcastle, UK), anti-8-hydroxy-2'-deoxyguanosine (8-OHdG) monoclonal antibody (Japan Institute for the Control of Aging, Nikken Seil, Shizuoka, Japan), rabbit anti-Fas ligand (FasL) polyclonal antibody (Nichirei) or mouse anti-granzyme B antigen monoclonal antibody (Nichirei). The antibodies cross-react with human materials according to the manufacturers' descriptions. Apoptosis was evaluated with the TUNEL method (Roche, Penzberg, Germany).

Results

The patient was diagnosed as having type 1 diabetes mellitus at the age of 19 years. She required haemodialysis at 34 years, and simultaneous pancreas–kidney transplan-

tation (SPK) was performed from a cardiac death donor at 36 years of age. The donor was a 32-year-old female who died of hypoxia. HLA typing of the donor was 2 and 33 for A loci, 44 and 51 for B loci and 4 and 13 for DR loci, whereas that of the recipient was the same for A and DR loci, but 44 and 55 for B loci (one antigen mismatch). Total ischaemic time was 8 h 49 min. Insulin therapy and haemodialysis were successfully discontinued postoperatively. Immunosuppressive drugs were administered with maintenance doses of tacrolimus 4 mg, mycophenolate mofetil 1,000 mg and prednisone 5 mg daily. Blood trough concentration of tacrolimus was 9.0 ng/ml and serum lipase was 81 U/l (normal range 16–51 U/l). The first pancreas biopsy was performed 9 months after the transplantation. The pancreas graft showed minimal chronic inflammatory infiltrate in the stroma (Drachenberg's grade II) and the kidney graft biopsy showed no signs of acute rejection. Beta cells were well preserved compared with the control (Fig. 1a–c) and no insulinitis was observed with CD3, CD4, CD8 and CD68 stainings (Fig. 1d–g). There were no Ki67-positive proliferating cells in the islets (Fig. 1h) but 8-OHdG staining, indicative of oxidative DNA damage, was diffusely positive in islet cells as well as exocrine tissue (Fig. 1i). Although normoglycaemia had continued, hyperglycaemia developed abruptly at 48 months after transplantation (fasting plasma glucose 15.4 mmol/l, HbA_{1c} 7.4%). Blood trough concentration of tacrolimus was in the therapeutic range (4.1 ng/ml) and serum lipase was 85 U/l. The graft biopsies revealed undetermined inflammation (Drachenberg's grade I) in the pancreas graft and minimal acute rejection (Banff's grade Ia) in the kidney graft. Regarding islet autoimmunity, anti-GAD antibody was undetectable before transplantation and at 24 and 42 months after transplantation, but had increased to 6.3 U/l by 48 months. Anti-insulinoma-associated protein 2 tyrosine phosphatase (IA-2) antibody was 18.5 U/l (the previous data were unavailable). Insulin secretion was severely suppressed (peak serum insulin in 10 g IVGTT, 271 pmol/l at the first biopsy, 95 pmol/l at the second biopsy), and intensive insulin therapy did not restore insulin secretion (serum C-peptide 6 min after glucagon i. v. injection, 0.66 nmol/l before treatment, 0.73 nmol/l after treatment). In the second pancreas graft biopsy (Fig. 2), insulin-positive beta cells were markedly reduced (Fig. 2a), and glucagon-positive alpha cells predominated (Fig. 2b). CD3-positive T lymphocytes (Fig. 2c), CD8-positive cytotoxic T lymphocytes (Fig. 2e) and CD68-positive macrophages (Fig. 2f) infiltrated around and into islets. No CD4-positive T lymphocytes were observed (Fig. 2d). The prevalence of islets infiltrated with more than three cells was 15 of 17 (88%) islets for CD3-positive T lymphocytes, 16 of 20 (80%) islets for CD8-positive T lymphocytes, and 15 of 18 (83%) islets for CD68-

Fig. 1 **a** Insulin staining in control pancreas. **b–i** The first pancreas graft biopsy performed at 9 months after transplantation. **b** Insulin staining. **c** Glucagon staining. **d** CD3 staining. **e** CD4 staining. **f** CD8 staining. **g** CD68 staining. Note glucagon (red) co-staining in **d–g**. **h** Ki67 staining. **i** 8-OHdG staining. Scale bar, 50 μ m



positive macrophages (Fig. 2g). Ki67-positive cells were evident within the islets (Fig. 2h) and the percentage of Ki67-positive cells in total islet cells was 1.5%. The 8-OHdG-positive cells were diffusely present in islets and exocrine tissue, but smaller, probably infiltrating, cells were negative for 8-OHdG (Fig. 2i). The infiltrating cells expressed FasL (Fig. 2j) as well as granzyme B (Fig. 2k). There were no TUNEL-positive cells in islet cells.

Discussion

We demonstrated recurrent type 1 diabetes mellitus associated with insulinitis in one patient under HLA-mismatch and immunosuppression. Acute allograft rejection was minimal in pancreas graft so the effects of allograft immunity on islet pathology seem to be negligible [2]. Recurrent type 1 diabetes mellitus is diagnosed by the presence of insulinitis and the selective loss of beta cells [1]. Reversal of autoimmune type 1 diabetes was reported in recipients of transplants from HLA-identical twins or siblings with no or minimal immunosuppression, whereas there was no recurrent diabetes with insulinitis in recipients of transplants from cadaver donors in early studies [1, 2]. Tydén et al. [4] were the first group to report that a 34-year-old recipient developed recurrent type 1 diabetes with seroconversion

of islet cell antibody and anti-GAD antibody 29 months after SPK with mismatched class I and II HLA and immunosuppression with ciclosporin, azathioprine and prednisolone. However, the recurring insulinitis in the transplanted pancreas remains to be characterised. The presence of insulinitis may be difficult to detect, in which case the selective loss of beta cells with intact alpha cells may indicate recurrent type 1 diabetes. According to this criterion, two more cases were reported under HLA-mismatch and immunosuppression [4, 7]. Increases in anti-GAD and IA-2 antibodies were reported in two of three patients. Until recently, recurrent autoimmunity after pancreas transplantation was considered rare under HLA-mismatch and standard immunosuppressive conditions. Recurrence of autoantibodies after pancreas transplantation was reported in a minority of recipients, and those with major increases in autoantibodies showed lower graft survival [8]. In addition to humoral autoimmunity, there were autoreactive CD4-positive T cells specific for GAD65 in peripheral blood of two recipients with recurrent hyperglycaemia [9]. It seems as if immunosuppressive therapy fails to prevent the recurrence of islet-specific autoimmunity, even though it prevents allograft immunity to the transplants in some recipients.

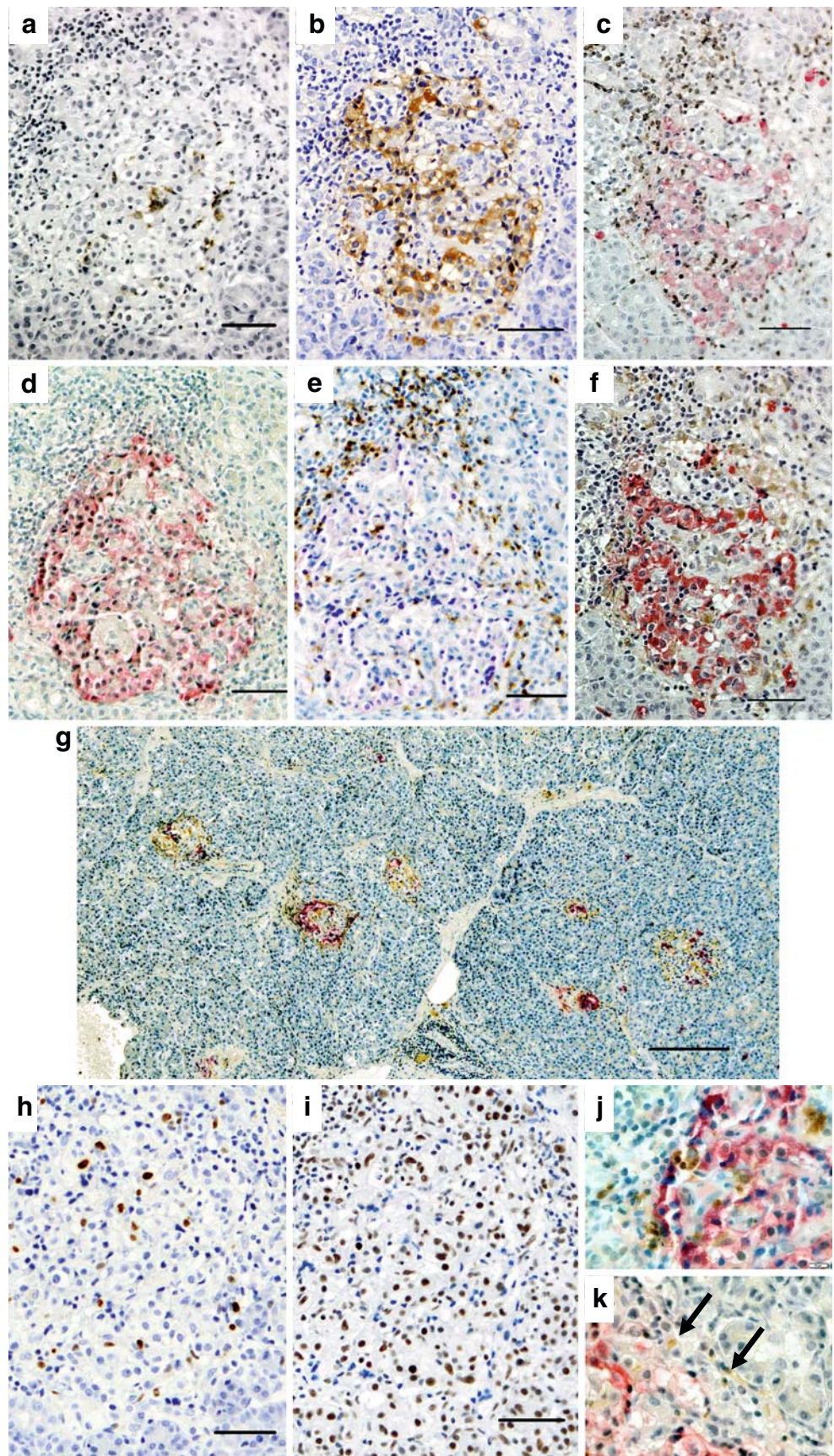
Although HLA plays a crucial role in the pathogenesis of autoimmune type 1 diabetes mellitus, experimental islet transplantation studies demonstrated that autoreactive

Fig. 2 The second pancreas graft biopsy performed at the development of hyperglycaemia 48 months post-transplant.

a Insulin staining. **b** Glucagon staining. **c** CD3 staining. **d** CD4 staining. **e** CD8 staining.

f, g CD68 staining. **h** Ki67 staining. **i** 8-OHdG staining.

j FasL staining. **k** Granzyme B staining. Note the same islet in **a–k** except **g** and the co-staining for glucagon (red) in **c, d, e, f, g, j** and **k**. Scale bar, 50 μ m (except **g**: scale bar, 200 μ m)



T cells destroyed islet transplants regardless of HLA matching in autoimmune diabetic NOD mice [10]. Antigen recognition seems to be processed via the host antigen-presenting cell-dependent (indirect) pathway. Fas–FasL and perforin–granzyme B are two major effector mechanisms of T cell-mediated cytotoxicity. Both mechanisms seem to be operative to kill beta cells also in transplanted pancreas. Moriwaki et al. [11] showed FasL-positive cells in the pancreas biopsies from patients with recent-onset type 1 diabetes, but did not detect TUNEL-positive cells, probably because of the very short time of completion of apoptosis. In the present study, cell replication in islets was observed only in association with insulinitis. Although there are no reports regarding beta cell replication in transplanted pancreas to our knowledge, our findings are in agreement with those of Meier et al. [12] who showed a more than 100-fold increase in the frequency of Ki67-positive beta cells in insulinitis. In addition, 8-OHdG-positive cells were diffusely present throughout the specimens probably because of the organ damage inherent in the transplantation procedure, including ischaemia–reperfusion injury. We have no explanation for the link between the cardiac death donor and the recurrent type 1 diabetes in our patient. However, a relationship seems to be unlikely because most recipients from marginal donors showed good survival of their pancreas graft [3].

In conclusion, the histological features of insulinitis in transplanted pancreas appeared to be consistent with common type 1 diabetes mellitus. However, the clinical course of the recurrence appeared to be more rapid, considering that type 1 diabetes mellitus usually has a long preclinical period.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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