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CASE REPORT

Association of cytomegalovirus infections with recurrence of humoral and cellular autoimmunity to islet autoantigens and of type 1 diabetes in a pancreas transplanted patient

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Summary

Association of type 1 diabetes and cytomegalovirus (CMV) is suspected and CMV infections have also been linked to increased risk of new onset post-transplantation diabetes. We monitored response to islet autoantigens, pancreatic endocrine function, and CMV infections in one type 1 diabetic patient receiving pancreas allograft. Time course analyses of levels of islet autoantibodies (Abs), IFN-γ ELISPOT response, analysis of T cell function, levels of C peptide together with CMV pp65 antigenaemia and viraemia and graft biopsy histopathology were performed in comparison with a cohort of diabetic recipients. Evidence of autoimmune activation to GAD and IA2, modification of $CD4^+$ CD25hi T cells, loss of pancreatic function, concomitantly with multiple CMV infections and allograft rejection with peri-insulitis is provided. The parallel between metabolic outcome, initiation and progression of autoreactivity to islet autoantigens and early CMV infections after transplantation, suggests that persistent CMV infections may be relevant to the pathogenesis of type 1 diabetes.

Introduction

Cytomegalovirus (CMV) is included amongst the environmental factors potentially relevant to the pathogenesis of type 1 diabetes [1,2]. Literature is scarce and contradictory and a limited number of studies analysed the role of CMV on the appearance and progression of islet autoimmunity. CMV infection has been also linked to the induction of new onset post-transplantation diabetes [3,4] as an independent risk factor. Post-transplantation diabetes is a common complication of organ transplantation, recognizing multiple causes, including the effects of immunosuppressive drugs, increasing age, ethnicity, post-transplantation weight gain [5]. The pathogenetic mechanisms underlying CMV-induced diabetes remain unclear, but several hypotheses have been discussed [6]. This association raises the possibility that CMV prophylaxis may be a useful and cost-effective strategy for management of new-onset post-transplantation diabetes.

Furthermore, CMV infections remain a major cause of morbidity and mortality in recipients of solid organ allograft and have been associated with increased rates of acute and chronic allograft rejection [7]. CMV productively infects all cell types involved in vascular rejection of solid organ transplantation. The allograft damage may depend on both direct and indirect effects of the virus [8].

In a scenario of a small number of studies about the possible connection between CMV and autoimmune or post-transplantation diabetes, we report herein a case of recurrence of autoimmunity to islet autoantigens and multiple CMV infections, with loss of endocrine function, in one type 1 diabetic patient receiving pancreas allograft.

Patients and methods

Clinical data

A 28-year-old Caucasian female type 1 diabetic patient with 14 years disease duration received pancreas allograft for brittle diabetes, severe neuropathy, proliferative retinopathy. BMI was 22.3 kg/m^2, C peptide was <0.5 ng/ml, creatinine clearance was 67 ml/min and HbA1c 9.9%. Patient also had autoimmune hypothyroidism.

Donor and recipient CMV IgG antibodies were positive. CMV pp65 antigenaemia (pp65 antigen-positive cells/200 000 leucocytes) and viraemia by CMV DNA quantitative real time PCR (DNA copies/ml of whole blood) [9] were monitored weekly in the first 2 months and every second week for the whole study period to detect infection at the very beginning. CMV pp-65 antigenaemia or detection of CMV DNA was classified as asymptomatic CMV infection or CMV disease, depending on the absence or presence of clinical symptoms or signs.

Immunological assays

Anti-GAD and anti-IA2 autoantibodies (Abs) were detected by radio-immunoassay (Medipan Gmbh and Euroimmun, Germany respectively). Cellular immune response to GAD antigen was assessed by IFN-γ ELISPOT analysis (U-Cytech, NL) as described [10]. As control population, 12 age-matched Caucasian healthy subjects without a history of diabetes were used. Mean spots/300 000 cells in test well were compared with mean of the background wells to derive a stimulation index (SI, ≥3 indicated a positive response) [10].

Three-colour flowcytometric analysis of suppressor function CD4$^+$ CD25hi T cells on freshly isolated PBMCs was performed using CELLQUEST software (BD Biosciences, I) [11]; monoclonal anti-CD4, anti-CD25, anti-DR45R0 (Caltag Laboratories, Burlingame, CA, USA), anti-HLA-DR and anti-CTLA-4 (BD PharMingen, San Diego, CA, USA) were used.

Findings of the index patient were compared with a cohort of 21 consecutive type 1 diabetic patients (mean age 41.5 ± 8 years, mean disease duration 28 ± 7 years) who received simultaneous pancreas–kidney (19 patients), pancreas–liver (1) or pancreas alone (1) transplantation, followed up for a mean of 39 months.

Case report

Surgical technique

The pancreas graft was implanted etherotopically in the right retro-peritoneal area, behind the right colon. Arterial inflow was provided by the right common iliac artery, while the venous outflow was constructed systemic, directly into the inferior vena cava. Drainage of the exocrine secretions of the graft was performed with an anastomosis between the duodenal graft and a jejunal loop of the recipient. No anti-viral prophylaxis was performed.

Immunosuppression

Induction of immunosuppression consisted of tacrolimus (0.075 mg/kg/bid), basiliximab (20 mg intra-operative and on day 4), methylprednisolone (500 mg intra-operative, 200 mg on day 1 and 50 mg on day 2) and mycophenolate mofetil (MMF) (2 g/day). Maintenance therapy consisted of tacrolimus (levels 8–10 ng/ml), MMF (2 g/day), prednisone (0.5 mg/kg/day) with a tapering to 5 mg/day. Insulin therapy was discontinued 1 month after transplantation.

Clinical course

CMV first appeared 24 days post-transplantation and patient started pre-emptive therapy with valganciclovir (1800 mg/day). During the first 52 weeks post-transplantation, the patients had 9 asymptomatic CMV infections and positive viraemia persisted for a total of 14 weeks (Fig. 1a) requiring treatment with valganciclovir or ganciclovir, depending on the viraemia, until repeated negative viraemia.

At 35 week post-transplantation, a first pancreatic graft biopsy showed acute rejection with increased amylase (127 U/l) and lipase (523 U/l) plasma levels. Pancreatic graft biopsy contained very few islets of Langerhans per section; however, haematoxylin–eosin staining revealed the presence of mononuclear cellular infiltrate surrounding some islets (Fig. 2), suggestive of peri-insulitis as described in non-obese diabetic mice [12] and recently in humans [13]. This coincided with asymptomatic CMV infection treated with ganciclovir. Rejection episode was first treated with methylprednisolone (500 mg/day for 3 days). Persistent acute rejection grade IV was evidenced by a second biopsy and treated with thymoglobulin (2 mg/kg/days for 12 days). Immunohistochemistry for CMV in tissue biopsies resulted negative. Concomitantly, C peptide level progressively decreased (Fig. 1b). The recurrence of type 1 diabetes was indicated by persistent loss of C peptide (<0.5 ng/ml) approximately 12 months after transplantation and by fulfilment of ADA criteria.

Immunological data

Before transplantation, ICA and GAD Abs were negative, IA2 Abs weakly positive (2 U/ml). Despite immunosuppression, after transplantation, the patient became

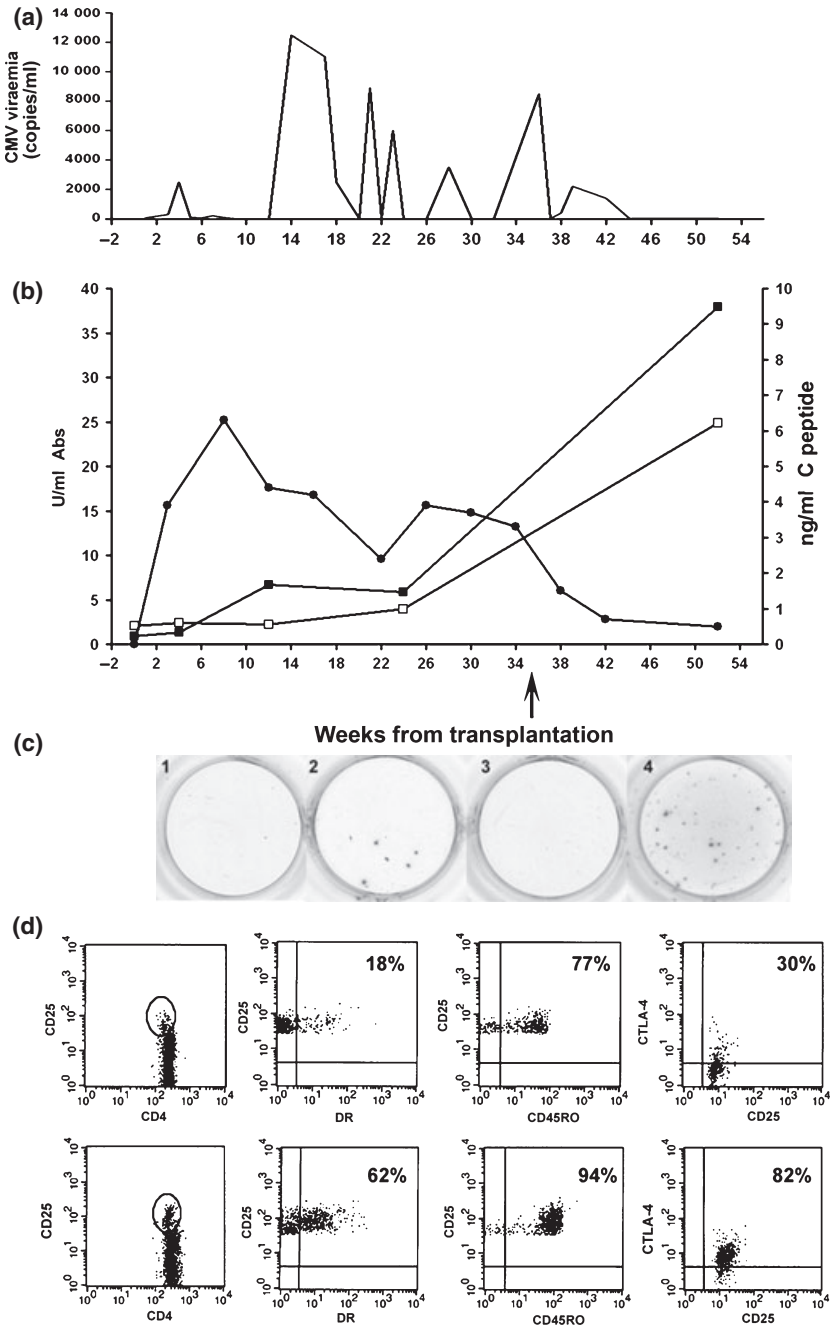

Figure 1 (a) Clinical course of cytomegalovirus (CMV) viraemia, expressed as DNA copies/ml, during the first 52 weeks after pancreas transplantation. (b) Time course of appearance and levels of anti-GAD (■) and IA2 (□) autoantibodies and of fasting C peptide (●) before and after pancreas transplantation. C peptide levels progressively decreased concomitantly to evidence of CMV infections. The arrow indicates the time of biopsy showing acute pancreatic graft rejection. (c) Representative IFN-γ ELISPOT response of patient's lymphocytes incubated with GAD before (2) and after pancreas transplantation (4) compared to corresponding background responses to vehicle alone (1 and 3). (d) Flowcytometric analysis of regulatory $CD4^+CD25hi$ T cells, coexpreesing HLA-DR and CD45R0 activation markers and intracellular CTLA-4 performed 1 year after transplantation in a representative diabetic patient with successful organ transplantation (top panel) compared to the patient with the recurrence of type 1 diabetes (bottom panel).

positive for GAD Abs 4 weeks after transplantation and the levels of both GAD and IA2 Abs progressively increased up to 34.9 and 24.9 U/ml respectively at week 52 (Fig. 1b).

IFN-γ ELISPOT response of patient's lymphocytes to GAD was negative before transplantation (SI 1.2) and became positive (SI 8.9) 1 year post-transplantation (Fig. 1c).

Flowcytometric analysis of suppressor function of $CD4^+$ CD25hi T cells showed a high proportion of the $CD4^+$ CD25hi population coexpressing HLA-DR (62%) and CD45R0 (94%) activation markers and a higher percentage expressing intracellular CTLA-4 (82%, MFI 9.2) compared with the normal population and nine diabetic recipients 1 year after successful pancreas–kidney transplantation (30% ± 14, MFI 4.7 ± 1.3) (Fig. 1d).

Eight of the 20 diabetic patients (40%) receiving simultaneous pancreas–kidney or pancreas–liver transplantation had only one asymptomatic CMV infection each and CMV viraemia became and remained negative after treat-

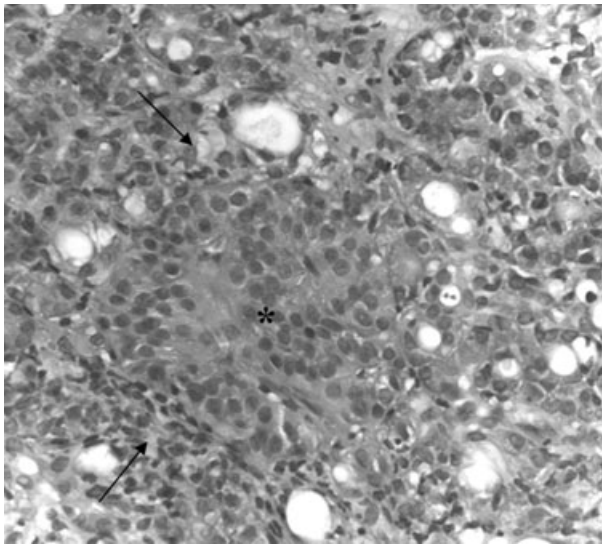

Figure 2 Haematoxylin–eosin staining of pancreatic graft biopsy specimen obtained at 35 weeks post-transplantation. The asterisk indicates a pancreatic islet and the arrows indicate the presence of inflammation infiltrate surrounding the islet. Original magnification: ×200.

ment. Fifteen recipients (75%) did not change their islet Abs status after transplantation, whereas five recipients became Abs negative, independently of the occurrence of CMV infection. All patients maintained insulin independence during the whole follow-up.

Discussion

Newly acquired and reactivated CMV infection has been linked to inflammatory-related processes in the human host, inducing vascular diseases and has been associated with increased rates of acute and chronic allograft rejection [7]. The pathogenetic mechanisms are largely unknown, but possibly involve both the effects of virus-encoded factors on the host immune system and the effects of the host immune response to the virus itself [8]. In particular, CMV infection of vascular cells induces proinflammatory adhesion molecules by action of IL-1β and chemokines [14], contributing to selective recruitment of leucocytes and leading to local enhancement of the inflammatory effects of CMV.

Furthermore, CMV is included amongst viruses suspected to be the environmental triggers of the development of β cells autoimmunity and type 1 diabetes [1,2]. Studies suggest also a link between CMV, as an independent risk factor, and new onset post-transplantation diabetes with impaired insulin release [3,4]. However, a causal relationship remains circumstantial.

The present case report adds new information on the potential CMV involvement in triggering islet autoimmunity. We evidenced a temporal relationship between CMV infection and loss of pancreatic endocrine function, suggesting that islet autoreactivity might be reactivated by repeated CMV infections, concomitantly with the acute allograft rejection. The documented parallel trend of the metabolic outcome, the initiation of islet autoimmunity and insulitis, and the repeated CMV infections, suggests a causal relationship. CMV infection/disease as well as repeated rejection episodes and recurrent autoimmunity all could be contributors to the graft failure. Primed, memory T lymphocytes recognizing islet autoantigens are resident in the lymph nodes of patients. Therefore, the requirements for activation of these autoreactive T lymphocytes, in terms of co-stimulation and antigen concentration, could be considerably lower than those needed to prime naive, alloreactive T cells. Therefore, the autoimmune response may be not susceptible to immunosuppression. Indeed, islet graft recipients and patients receiving pancreas transplantation develop evidence of islet autoreactivity, despite appropriate immunosuppression [15,16].

The patient also developed a pattern of regulatory T cells similar to that described in type 1 diabetic patients at onset [11]. However, it cannot be excluded that this population represents activated effector memory T cells described at type 1 diabetes onset [17] or a balance of both activated effector and regulatory T cells [11].

Notably, the graft biopsy revealed, concomitant with acute rejection, the presence of cellular infiltrate concentrated around the periphery of some islets, compatible with peri-insulitis, as seen at early stage of disease progression. This is highly reminiscent of the spatial distribution of leucocytes in the insulitis associated with β cell destruction, described in human type 1 diabetes [13], where most leucocytes surround the islets and extend into spaces between the exocrine glands.

This report adds to the description of recurrent insulitis and autoimmune β cell destruction in one pancreatic allograft with a predominant fraction of infiltrating T cell reactive to CMV [18]. Furthermore, there is evidence of generation of autoantibodies directed to the islets in mice undergoing CMV infection [19].

Several hypotheses have been proposed to explain the link between CMV and islet autoimmunity [6]. These include direct viral destruction of β cells, induction of virus-specific cytotoxic T cells killing virus-infected β cells, enhancement of antigen presentation, bystander activation of autoreactive T cells caused by the inflammatory environment during infection, and destruction by cross-reacting T cells. Notably, GAD65-specific T cells cross-react with a peptide of the major DNA-binding

protein of CMV, suggesting a mechanism of molecular mimicry [20].

Further studies are warranted to elucidate a causal relationship between CMV infection and type 1 diabetes.

Authorship

MMZ, EF: Designed, performed study, analysed data and wrote paper; FG, RR, ED: Performed study; RQ, IM: Collected data; PCP, MS: Analysed data; GC: Analysed data and wrote paper.

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