Cytomegalovirus Prevalence and Transmission After Islet Allograft Transplant in Patients with Type 1 Diabetes Mellitus

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Cytomegalovirus (CMV) serological status of transplant donors and recipients has important implications on antiviral prophylaxis, morbidity/mortality, donor selection and hospital stay. We evaluated CMV prevalence in our islet transplant candidates (ITC) in comparison with organ donors. We correlated the CMV serological status of our ITC with serology for Epstein-Barr virus and Parvovirus B19, auto-antibodies, patient’s age, age at DM onset, duration of DM, gender, race, ABO group, HLA haplotype and C-peptide levels. Cytomegalovirus transmission after islet transplant using the Edmonton regimen was also evaluated. Cytomegalovirus seropositivity varied according to patient group, age, gender and race. Type 1 DM patients had reduced odds of CMV seropositivity when compared with organ donors. In all groups studied, older patients, females, and non-Caucasians were more likely to be CMV seropositive. In addition, no CMV reactivation, infection or disease was observed among our transplanted patients using this steroid-free regimen even after donor/recipient CMV mismatch.

Key words: Cytomegalovirus, islet transplant, type 1 diabetes mellitus

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Introduction

Cytomegalovirus (CMV) (human herpersvirus 5) is a prevalent pathogen among the general population, with prior exposure shown by positive serology in 40–100% of subjects (1). It is an important symptomatic infectious disease, accounting for significant morbidity. Cytomegalovirus has been detected in most body tissues and remains latent in principal reservoirs such as the white blood cells (lymphocytes and monocyte) and CD 13-positive cells (2). It is among the most frequent symptomatic infection occurring in transplant recipients, with a reported incidence of 20–60%. Cytomegalovirus disease can occur any time after transplantation, but is most common 1–4 months postoperatively, with evidence of active viral replication ranging from 50 to 75%. Transmission of CMV has been observed after bone marrow, solid organ (3) and islet transplantation in islet after kidney (4) and simultaneous islet kidney transplantation (4,5). In solid organ transplantation, CMV is transmitted from seropositive organ donors to seronegative recipients, even in the presence of prophylaxis. The impact of potent immunosuppressive regimen on CMV infection is well documented in solid organ transplantation (6–8). Important risk factors for developing CMV disease include re-transplantation and the use of lymphocyte-depleting antibodies for immunosuppression (6). Cytomegalovirus infection increases the risk of rejection and predisposes the immunosuppressed recipient to opportunistic infections (9), which ultimately increase hospitalization and cost (10).

A retrospective analysis of Kidney Pancreas Transplant (KPT) recipients has shown that antiviral prophylaxis results in delayed CMV onset and reduced severity of disease (3). Cytomegalovirus infection and invasive disease were reported in 22% of patients, with 12% showing infection and 10% CMV disease after 3–6 months of prophylaxis. The highest incidence of CMV infection (44%) was found in the D+/R– group vs. other serological groups (17%). The highest rejection was also seen in D+/R– group when compared with other groups (25% vs. 7%). The D−/R− group showed no CMV infection and had the best outcome in terms of decreased rejection and graft loss (3). Other Simultaneous Pancreas Kidney Transplant (SPKT) studies have shown similar results with disease occurrence, depending on the donor/recipient CMV status: 0% for D−/R+, 2.8% for D−/R−, 25.6% for D+/R+, and 40.6% for D+/R− (6).

Few studies have analyzed CMV and islet alone transplantation in patients with type 1 DM. In the initial series of patients reported by the Edmonton group no CMV infection was observed, despite four recipients being seronegative.
for CMV before transplantation and receiving an allograft from seropositive donors (11).

In the present study, we evaluated the CMV serological status of our islet transplant candidates (ITC) as well as our transplanted patients, and the predictive relative risk of infection, by comparing the results with organ donors and other studies. We additionally evaluated whether factors such as age, gender, race, HLA haplotype, islet autoantibodies to tyrosine phosphatase (IA-2) and glutamate decarboxylase (GAD–65) presence/absence, basal C-peptide levels and ABO blood group were associated with CMV seropositivity in the populations of ITC and organ donors.

Materials and Methods

Patients
Three hundred and sixty-two patients age 18–65 years with type 1 DM seeking acceptance to our islet transplantation program (from urban and rural areas throughout the United States) were evaluated. The mean age was 41.3 ± 9.5 years and the female/male ratio was 196/160 (1:23). The demographic characteristics of the subjects are outlined in Table 1. All patients were seen at the Diabetes Research Institute from 1995 to 2004 as ITC. These candidates had CMV serologies, stimulated C-peptide, psychologi- cal evaluation, routine laboratory, medical history and physical examination performed following protocol guidelines. All participants signed a consent form approved by our Institutional Review Board.

The control group consisted of organ donors (n = 1042), representative of the general population. The mean age was 39.7 ± 13.4 years and the female/male ratio was 385/659 (0.70) (Table 1). A randomized control group of patients with type 1 DM was difficult to establish because the majority of self referred candidates belonged to varying socioeconomic status, diverse ethnic groups and resided in different states.

CMV, EBV and parvovirus laboratory evaluation
Serum antibodies to CMV (IgM and IgG) were measured by enzyme-linked immunosorbent assay (Dade Behring Enzygnost, Deerfield, IL, USA). Cytomegalovirus serological status was correlated with age, race, at onset of type 1 DM, duration of type 1 DM, basal C-peptide, gender, HLA haplotype (A1, A2, B1, B2, BW1, BW2, CW1, CW2, DR1, DR2), ABO group, Epstein-Barr virus (EBV; IgM and IgG) antibodies (Dade Behring Enzygnost), B19 (IgM and IgG) antibodies (Dade Behring Enzygnost), GAD–65 (Diabetes Research Institute, Miami, FL, USA) and IA-2 autoantibodies (Diabetes Research Institute). Cytomegalovirus polymerase chain reaction (PCR) (Eurogentec, San Diego, CA, USA) was performed following the manufacturer’s instructions. Cytomegalovirus PCR was performed using blood at weekly intervals post-transplant for 4 months, every 2 weeks up to 6 months, monthly up to 12 months, and then every 3 months. In addition, CMV serology was performed every 3 months. In four patients studied under a different protocol, no CMV PCR was performed. However, CMV serology was measured at 2-week intervals post-transplant for 3 months and every month thereafter.

Ilet preparation and transplantation
Islets were isolated from 56 human pancreata obtained from multorgan donors. Signed donor consent was obtained from living relatives by the Organ Procurement Organizations. Islets were isolated utilizing the automated
CMV Prevalence and Transmission in Type 1 DM Patients

A digestion-filtration method (12) followed by density gradient purification in a COBE 2991 centrifuge (COBE, Lakewood, CO).

The percutaneous transhepatic approach was used to gain portal vein access using ultrasound and fluoroscopic guidance (13). Once access was confirmed, a minimum of 5000 islet equivalent (IEQ)/kg were infused using the bag method (14).

Antiviral and immunosuppressive therapy
A steroid-free immunosuppressive regimen similar to that described in the Edmonton Protocol was utilized. Immunosuppression consisted of induction with daclizumab [Zenapax, Roche, Nutley, NJ, USA] 1 mg/kg intravenously × 5 doses; the first dose at the time of transplant and the remaining doses at 14-day intervals. Nineteen of 29 islet recipients received monthly intravenous infusions of daclizumab 1 mg/kg for the first year post-transplant. Tacrolimus (Prograf, Fujisawa, Deerfield, IL, USA), 1 mg was given orally before transplant, then 1 mg twice daily to maintain serum levels of 3–6 ng/mL (IMX, enzyme immunoassay, Abbott). Sirolimus (Rapamune, Wyeth-Ayerst, Madison, NJ, USA), was given orally at an initial pretransplant dose of 0.2 mg/kg. The dose was then adjusted to attain 24-h trough blood levels of 12–15 ng/mL (HPLC) for the first 90 days, and 7–10 ng/mL thereafter. Valganciclovir (Valcyte, Roche; 450 mg twice a day; n = 18) or Ganciclovir (Cytovene, Roche; 1000 mg three times a day; n = 11) were given orally for 3 months. Pentoxifylline (Trental, Aventis, Bridgewater, NJ, USA) 400 mg was given orally three times a day for 3 months.

Definition of infection
Patients were considered CMV infected when antibodies were detected or positive CMV DNA was demonstrated using PCR. In addition, CMV disease is considered if prolonged episodes of unexplained fever with constitutional signs and symptoms, and abnormal laboratories (leucopenia, thrombocytopenia, elevated liver enzymes) are observed. Cytomegalovirus disease is confirmed with viral isolation, viral load testing (quantitative CMV PCR) and the demonstration of CMV antigenemia.

Statistical analysis
We evaluated each potential predictor for its bivariate association with CMV serology using chi-squared tests. We additionally evaluated the bivariate association between CMV serology and patient group (patients with type 1 DM, transplant donors). Multivariate logistic regression models were used to analyze the data. In these models, binary outcome of CMV serology (positive or negative) was evaluated for its association with each potential predictor of interest adjusted for levels of all other predictors. Using this multiple logistic regression model, we were additionally able to assess differences in CMV serology between groups of patients.

In order to evaluate reduction in risk of CMV transmission/disease among transplant recipients, we analyzed data using a series of exact one sample test for binomial proportions. One-sided tests of significance were constructed to detect a reduction in CMV risk from a null risk ranging from 20% to 60% (Table 2).

Table 2: Statistical significance of observed rates of cytomegalovirus transmission among donors and recipients

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>n</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive donor to negative recipient</td>
<td>12</td>
<td>0.0687</td>
<td>0.0138</td>
<td>0.0022</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Positive donor to positive recipient</td>
<td>10</td>
<td>0.1074</td>
<td>0.0282</td>
<td>0.0060</td>
<td>0.0010</td>
<td>0.0001</td>
</tr>
<tr>
<td>Positive donor (either recipient)</td>
<td>22</td>
<td>0.0074</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Negative recipient (either donor)</td>
<td>16</td>
<td>0.0281</td>
<td>0.0033</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Entire population</td>
<td>29</td>
<td>0.0015</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Results

Preliminary analysis
The prevalence of positive CMV serology in patients with type 1 DM was 38%, which was significantly lower than the 67% value observed in organ donors (p < 0.0001) (Figure 1A). By contrast, positive EBV serology was recorded in 98% of type 1 DM patients (n = 327) and B19 positive serology in 64% (n = 56) (Figure 1B), values non-significantly different from those seen in the general population (15,16). Patients with type 1 DM had an average age of 41.3 years, which was slightly, but significantly, greater than the average age of organ donors (39.7, p = 0.01) (Table 1). In both groups, age was associated with CMV positivity. Bivariate analysis revealed that the mean age of CMV-positive patients over both groups was 41.2 years,
which was significantly higher than the mean age of CMV-negative patients (38.5, p < 0.0001). Significant bivariate associations additionally existed between gender and CMV serology. Over both groups, 61% of females were CMV positive, which was significantly greater than the observed positivity in males (55%, p = 0.03). Race (Caucasian, non-Caucasian) was found to be significantly associated with CMV serology. Over the two groups, 48% of Caucasians were CMV positive, which was significantly less than the observed positivity in the non-Caucasians (76%, p < 0.0001). Among patients with type 1 DM, 41% were mothers (n = 81), 75% were married couples (n = 178), and 25% were single patients (n = 60). Cytomegalovirus seropositivity increased with a parity of: 1 = 33%, 2 = 43% and ≥3 = 62% (Table 1). ABO and Rh classifications were not significantly related to CMV serology in the patient groups studied. Among patients with type 1 DM, age of diabetes onset was also significantly related to CMV positivity. The mean ages of diabetes onset were 13.6 and 15.7 years for patients negative and positive for CMV serology, respectively (p = 0.03). Duration of diabetes was not found to be significantly related on the bivariate level with CMV serology in this group, nor was there any significant associations with positivity of IA-2, GAD-65, EBV or B19. No association was observed for HLA (A1, A2, B1, B2, BW1, BW2, CW1, CW2, DR1, DR2) antigens or basal C-peptide with CMV, EBV or B19.

**Logistic regression analysis**

Results from multiple logistic regressions, illustrated in Table 3, revealed CMV serology was significantly associated with gender, age, race, and patient group. The odds of CMV positivity were 1.47-fold greater for females than males of the same age and patient group (p < 0.0001). The odds of CMV positivity were 1.24-fold greater for a patient in the same patient group and of the same gender (p < 0.0001). Non-Caucasians had significantly greater odds of CMV seropositivity compared with Caucasians of similar age, gender and patient group (OR = 2.92, p < 0.0001). After adjusting for age, gender and race, patient group was also found to be significantly associated with CMV positivity. The odds ratio and corresponding p-value for CMV positivity comparing type 1 DM patients with organ donors of similar race, gender, and age was 0.35, p < 0.0001. Thus, even after adjusting for the potentially confounding effects of age, race, and gender the presence of type 1 DM seemed to have a negative association with CMV positivity. Among patients with type 1 DM, no other factor studied was found to be significantly associated with CMV positivity. Although age of onset seemed to be relevant from a preliminary bivariate analysis, this association probably captured the underlying significant effect of age on CMV positivity.

**CMV transmission in islet transplant patients**

A total of 29 patients with type 1 DM underwent islet transplantation at our institution (Table 1). Forty-nine infusions were performed using the islets obtained from 56 pancreata. Donor CMV positivity was 48% (n = 56) pretransplantation.

The pretransplantation CMV status of our islet recipients was 45% positive (n = 13) and 55% negative (n = 16). The CMV status of the transplanted donor/recipient subgroups was: D+/R−: 41% (n = 12); D+/R+: 35% (n = 10); D−/R+: 10% (n = 3); and D−/R−: 14% (n = 4). Patients receiving islets from at least one CMV-positive donor were included in the D+ groups for analysis. In the group D+/R− (n = 12) nine patients received islets from one donor, two from two donors and one from three donors.

No CMV transmission, reinfection, reactivation or invasive disease was observed by CMV serology, PCR and clinical evaluation after islet transplantation (n = 29) (followed up to 450 days). A total of 485 CMV PCR and 128 CMV serologies were performed in 25 patients post-transplant and all yielded CMV negativity. A total of 51 CMV serologies were performed in four patients from a different trial and showed negative results.

Table 2 illustrates the statistical significance of these findings for one-sided tests of reduction in CMV risk from a range of infection risks of 20–60%. Thus, each entry in this table represents the p-value corresponding to the statistical significance of the observed rate of infection (0) for a one-sided test where the null hypothesis is that the true infection rate is 20–60% (depending on the table column).

Table 3: Logistic regression results

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 DM vs. organ donor*</td>
<td>0.35</td>
<td>(0.25, 0.49)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender (female vs. male)*</td>
<td>1.47</td>
<td>(1.13, 1.89)</td>
<td>&lt;0.0032</td>
</tr>
<tr>
<td>Age (10-year increase in age)*</td>
<td>1.24</td>
<td>(1.12, 1.38)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Race (non-Caucasian vs. Caucasian)*</td>
<td>2.92</td>
<td>(1.84, 4.62)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Race (unreported vs. Caucasian)</td>
<td>1.23</td>
<td>(0.92, 1.66)</td>
<td>0.15</td>
</tr>
<tr>
<td>10-year increase in DM duration among Type 1 DM</td>
<td>0.99</td>
<td>(0.80, 1.52)</td>
<td>0.28</td>
</tr>
<tr>
<td>IA2 (positive vs. negative) among Type 1 DM</td>
<td>0.94</td>
<td>(0.54, 1.66)</td>
<td>0.85</td>
</tr>
<tr>
<td>GAD65 (positive vs. negative) among Type 1 DM</td>
<td>0.98</td>
<td>(0.53, 1.80)</td>
<td>0.50</td>
</tr>
<tr>
<td>ABO blood group (A, B, or AB vs. O)</td>
<td>1.15</td>
<td>(0.85, 1.55)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*Statistically significant at α = 0.05.
and the alternative hypothesis is that there is a reduction in the rate of infection. p-values <0.05 represent tests where the null hypothesis would be rejected in favor of the alternative (reduction in infection risk).

These findings support statistically significant (\( \alpha = 0.05 \)) reductions in CMV risk from 30% or greater for each combination of donor/recipient serostatus, and statistically significant risk reductions from 20% when examining all transplants using positive donors, negative recipients, or the combined population.

**Discussion**

We have observed a lower percentage of positive serology for CMV in a cohort of 362 patients with type 1 DM, when compared with organ donors as representative of the general population. This is not a result of a generalized unresponsiveness towards viral antigens, as the serological status of our study population to other common viruses (parvovirus and EBV) mimics the general population (15,16).

Some studies have correlated CMV seropositivity with low socioeconomic status, increased parity, race and older age. Our patient population represents a heterogeneous group, which is the result of a diverse range of socioeconomic strata and race living in rural and urban areas throughout the USA. Actually, only 35% of our ITC resides in Florida. It comprised adults ranging in age from 18 to 65 years (mean age 41.3 ± 9.5), including mothers (41%; parity of: 1 = 14%, 2 = 14%, and \( \geq 3 = 13% \)), married couples (75%), and single patients (25%). We had to assume that these subjects had been exposed to risk factors for development of CMV antibodies. The prevalence of CMV seropositivity increased with parity as expected from 42% in nulliparous women to 62% in women with three or more children. Eighty-nine percent of our ITC (in whom data was available) were Caucasians in accordance with race distribution in type 1 DM. A similar racial distribution has been reported in the patients with type 1 DM receiving SPKT (3,6,8; 89%, 82% and 84%, respectively). The mean ages for these patients were 40, 37 and 39 years, respectively.

Published CMV prevalence in the Caucasian population is reported as 21% to 24%, for ages 18–22 years (17), and 47% in Caucasian women of middle/upper socioeconomic status older than 25 years (18). In our study, 38% of our ITC were CMV positive. The observation that the presence of antibodies against CMV is less frequent in subjects with type 1 DM has also been reported in SPKT (3,6,8).

Kaufman et al., in a randomized multicenter prospective study in 18 pancreas transplant centers in the United States (8), reported a CMV seropositivity of 33% (n = 174). Frank et al. (19) recently reported in AJT 40% CMV seropositivity in 9001 recipients of KPT reported to UNOS from 1987 to 3/25/2002. Other studies (3,6) have reported a CMV seropositivity of 55% (n = 74) and 32% (n = 100), respectively.

Also, our observation of a negative correlation between additional autoimmune diseases and CMV serology (20) may suggest the provocative hypothesis that CMV infection might exert a protective/negative role on the development of autoimmunity. Further studies are warranted.

The second interesting finding of our study is that the islet transplants performed in Miami have not resulted in new infection by CMV or reactivation of the virus in patients with type 1 DM. This observation is in keeping with that reported by the Edmonton group utilizing a largely identical steroid-free immunosuppressive regimen. They reported no CMV infection, despite four patients being seronegative for CMV and receiving islets from seropositive donor (11). While seroconversion and infection are common occurrences in recipients of solid organs, it appears that islet transplantation is associated with a negligible risk of CMV transmission and/or reactivation. This might be due to the low amount of tissue that is transplanted in islet recipients, resulting in an insufficient viral load to cause transmission or disease. Not only the tissue is quantitatively different from that of solid organ transplants, but also there might be qualitative differences (i.e. the mass of passenger leukocytes), which could explain the observed outcome. There might be additional variables that contribute to explain the observed results, as, for example, in the Giessen experience, it was reported that seroconversion/CMV disease was a common occurrence in CMV+/CMV− donor-recipient combinations in islet after kidney transplants. This might reflect differences in the immunosuppressive regimens utilized in the different studies, as well as differences in CMV prophylaxis and the use of steroids. It was reported, in fact, that the use of T-cell-depleting agents increases the risk of CMV transmission in solid organ recipients, and such an agent was indeed utilized in the Giessen trial. Our protocol and that from Edmonton do not include T-cell-depleting agents for recipient induction, but rather a T-cell activation blocker (Daclizumab).

Taken collectively, our observations suggest that a negative correlation between CMV serology and type 1 DM existed in the clinical population examined. The reasons for this significant difference from the general population need to be understood. Further, and importantly, there is no CMV transmission in islet recipients, possibly in virtue of: (a) the small mass of tissue that is transplanted; (b) the characteristics of the immunosuppressive regimen utilized; and (c) the efficacious antiviral prophylaxis.

Therefore islet transplantation appears safe from the standpoint of CMV transmission risk (despite the majority of ITC will belong to the group D+/R−, D+/R+), although interpretation of the risk for transmission/reactivation of CMV can only be made in light of ongoing antiviral prophylaxis.
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