

An Assessment of Donor-to-Recipient Transmission Patterns of Human Cytomegalovirus by Analysis of Viral Genomic Variants

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Background. We studied human cytomegalovirus (CMV) donor-to-recipient transmission patterns in organ transplantation by analyzing genomic variants on the basis of CMV glycoprotein B (gB) genotyping.

Methods. Organ transplant recipients were included in the study if they had CMV viremia, if they had received an organ from a CMV-seropositive donor, and if there was at least 1 other recipient of an organ from the same donor who developed CMV viremia. Genotypes (gB1–4) were determined by real-time polymerase chain reaction.

Results. Forty-seven recipients of organs from 21 donors developed CMV viremia. Twenty-three recipients had a pretransplant donor/recipient (D/R) CMV serostatus of D⁺/R⁺, and 24 had a serostatus of D⁺/R⁻. The prevalences of genotypes in recipients were as follows: for gB1, 51% ($n = 24$); for gB2, 19% ($n = 9$); for gB3, 9% ($n = 4$); for gB4, 0% ($n = 0$); and for mixed infection, 21% ($n = 10$). Recipients of an organ from a common donor had infection with CMV of the same gB genotype in 12 (57%) of 21 instances. Concordance between genotypes was higher among seronegative (i.e., D⁺/R⁻) recipients than among seropositive (D⁺/R⁺) recipients, although discordances resulting from the transmission of multiple strains were seen. In seropositive recipients, transmission of multiple strains from the donor could not be differentiated from reactivation of a recipient's own strains.

Conclusion. Our analysis of strain concordance among recipients of organs from common donors showed that transmission of CMV has complex dynamic patterns. In seropositive recipients, transmission or reactivation of multiple CMV strains is possible.

Cytomegalovirus (CMV) remains a significant pathogen in solid-organ transplant recipients [1, 2]. Several studies have identified pretransplant donor/recipient (D/R) CMV serostatus as the main risk factor involved in the development of CMV infection after transplant. The highest incidence of CMV disease occurs among D⁺/R⁻ patients (primary infection), followed by D⁺/R⁺ pa-

tients [3–5]. Theoretically, in the D⁺/R⁺ setting, the infecting CMV strain can originate either from the donor organ (superinfection) or as a consequence of the reactivation of the recipient's own strain (reactivation). The majority of studies conducted to date have suggested that the main source of CMV in this setting is the transplanted organ [6–10], mainly on the basis of strain analysis using restriction profiles. However, the potential limitations of these studies include the low sensitivity of the methods used (especially to distinguish between different strains and to detect multiple strains in the same sample) and the lack of viral quantification. Given that major changes in immunosuppressive and antiviral regimens have occurred since the publication of these studies, it is unknown whether the predicted transmission patterns remain valid.

Most efforts to study CMV gene polymorphism have focused on envelope glycoproteins because they are targets for neutralizing antibodies, can induce strain-specific antibodies, and are involved in virus entry and cell-to-cell spread [11, 12]. CMV glycoprotein B (gB),

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Table 1. Primers and probes used to determine glycoprotein B (gB) genotypes.

Type, primer/probe polarity	GenBank accession no.	Sequence (5'→3')	Length (location), nt	Size of amplicon, bp	Use
gB1 (HS5GLYBG)	M60929			72	Genotyping
Forward primer		CATACGACGCTGCTGCTCACT	22 (121–142)		
Reverse primer		GCTGACCGTTTGGGAAGAAG	20 (144–168)		
Probe		TCGATCCGGTTCAGTCTCT	19 (173–192)		
gB2 (HS5GLXBI)	M60931			79	Genotyping
Forward primer		TCTTTGGTGAATTGGAACGT	21 (1312–1332)		
Reverse primer		TGCTACTCGTACTTCTTCTGGTCTA	26 (1365–1390)		
Probe		ATCCAGTCTGAATATCA	17 (1334–1363)		
gB3 (HS5GLYBM)	M85228			72	Genotyping
Forward primer		TGTTGGAAC TGAACGTTTGG	21 (1316–1336)		
Reverse primer		TGCCCGTACTTCTTCTGGTTCT	22 (1366–1387)		
Probe		CGGTGTGAACTCCA	14 (1340–1364)		
gB4 (HS5GLYBM)	M60926			75	Genotyping
Forward primer		AAACGTGTCCGCTTCGAAACT	22 (1242–1263)		
Reverse primer		TCCACCAGAGATTTTTGCTTGA	22 (1295–1316)		
Probe		CCGGCGGACTAGTAGT	16 (1265–1290)		
CMV-PCR1 and 2 gpB518, reverse primer	M60929	ATAGGAGGCGCCACGTATTC	20 (499–518)	...	Sequencing
CMV-PCR1 gpB265, forward primer	M60929	TACCCTATCGCGTGTGTTT	20 (265–284)	254	Sequencing
CMV-PCR2 gpB212, forward primer ^a	M60929	CATACGACGCTGCTGCTCACT	22 (121–142)	397	Sequencing

NOTE. PCR, polymerase chain reaction.

^a Primer sequence was the same as for gB1 (HS5GLYBG).

encoded by *UL55*, contains a variable region encompassing the protease cleavage site. Heterogeneity in this region allows the partition of strains into 4 gB genotypes. In the immunocompromised host, individual gB genotypes have been inconsistently associated with some differences in clinical manifestations or organ tropism [13–15]. In a previous study, we developed a novel, multiplexed, real-time polymerase chain reaction (PCR) assay for the simultaneous detection and quantification of CMV gB genotypes [16]. We have now used this sensitive and quantitative assay to study the epidemiology of donor-to-recipient CMV transmission after transplant.

The aim of the present study was to assess donor-to-recipient transmission patterns by analyzing CMV genomic variants in recipients of organs from common donors. Correlations between transmission patterns and clinical or virologic outcomes were investigated.

METHODS

Study population. All CMV-seropositive donors who donated >1 organ between January 2000 and April 2006 at the University of Alberta Hospital, Edmonton, Alberta, Canada, were screened for the study. Organ transplant recipients were included in the study if they developed CMV viremia, if they received an organ from a CMV-seropositive donor, and if there

was at least 1 other recipient of an organ from the same donor who also developed CMV viremia. The study was approved by the local institutional review board. Definitions of infectious diseases developing after transplant followed the American Society of Transplantation recommendations for the screening, monitoring, and reporting of infectious complications in recipients of organ transplants during immunosuppression trials [17].

Immunosuppressive and antiviral regimens. The immunosuppressive regimens used have been described elsewhere [18]. Briefly, the standard immunosuppressive regimen for all organ transplant recipients consisted of tacrolimus, mycophenolate mofetil, and prednisone. The dose and duration of the prednisone treatment varied among the transplant recipients. Induction therapy consisting of polyclonal antibodies was given for delayed graft function or to highly sensitized (i.e., those with a high panel reactive antibody score) kidney transplant recipients. All heart and lung transplant recipients received induction therapy with antithymocyte globulin.

In all recipients of nonlung transplants, oral ganciclovir or valganciclovir was used for CMV prophylaxis. All CMV mismatched (D^+/R^-) recipients received oral ganciclovir (1000 mg 3 times a day) or valganciclovir (900 mg once daily) after the transplant for 14 weeks. All other donor-recipient subgroups were managed with preemptive therapy. Patients were also rou-

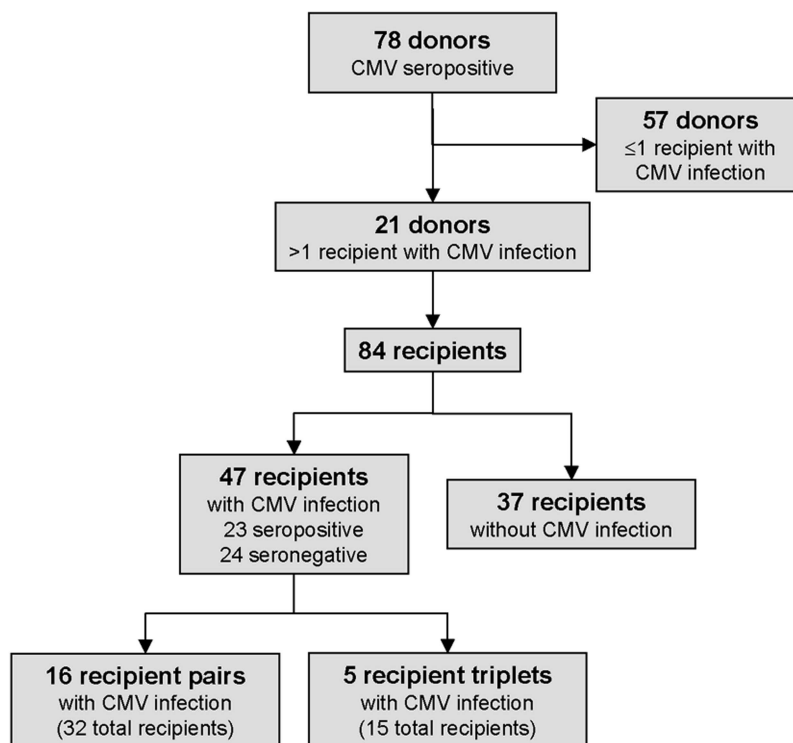


Figure 1. Cytomegalovirus (CMV) infection in recipients of transplanted organs from common donors.

tinely given intravenous ganciclovir for CMV prophylaxis if they received monoclonal or polyclonal antibody therapy for the duration of treatment of rejection episodes. Lung transplant recipients other than D^-/R^- patients received intravenous ganciclovir prophylaxis for at least 2 weeks after transplant before stepping down to oral therapy.

Real-time genotyping by quantitative PCR. Real-time, simultaneous gB genotyping and viral load determination was done using methods that have been described elsewhere [16]. Briefly, primers and probes targeting the CMV gB1, gB2, gB3, and gB4 genes were designed on the basis of the variable region of the gB gene by means of Primer Express 3.0 (Applied Biosystems). To enhance the specificity and sensitivity of the genotyping probes, the gB1, gB2, gB3, and gB4 probes were designed as 3' minor groove binder probes and labeled with the TaqMan dye 6-carboxyfluorescein, VIC, NED, or Cy5, respectively (Applied Biosystems). Primer and probe sequences are summarized in table 1. Double-stranded CMV DNA was extracted from 200 μL of plasma by means of the Qiagen DNA Mini Kit, in accordance with the manufacturer's protocol, and was eluted from the column using 50 μL of dH_2O . Real-time PCR was done in a closed tube system using the ABI Prism 7500 Sequence Detection System (Applied Biosystems). The reaction was performed in a 25- μL volume containing 12.5 μL of Universal DNA Master Mix (Applied Biosystems), 5 μL of DNA, 400 nmol/L each primer, and 200 nmol/L probe (for gB1, gB2, gB3, and gB4). After an initial incubation at 50°C for 2 min to activate uracil-N-

glycosylase and then an incubation at 95°C for 10 min for denaturing, PCR amplification was performed with 45 thermal cycles of 94°C for 20 s and 60°C for 1 min after reheating at 95°C for 5 min. Amplification data were collected and analyzed with ABI 7500 System SDS software (version 1.4; Applied Biosystems). A known copy number of a DNA fragment (1500 bp) amplified from strain AD169 was diluted in a 10-fold series (1 to 1.0×10^7 genome copies) and used to set up an external standard curve for the quantitation of gB genotype-specific viral loads. The limit of detection for gB1, gB2, gB3, and gB4 was 100 copies/mL. To compare the CMV load for each gB genotype, we used the highest viral load measured by real-time quantitative PCR before antiviral therapy was started in patients with CMV disease or asymptomatic CMV infection.

Sequencing of CMV strains. Two PCR products with 254 and 397 bp located at the 5' end of gB were amplified using the primers shown in table 1. The PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen) and were sequenced in 2 directions by means of the same forward and reverse primers, using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and an ABI Prism 3100 sequencer (Applied Biosystems) in accordance with the manufacturer's instructions. Primary sequence data were assembled, and the consensus sequence from each amplicon was generated using Lasergene software (version 6.0; DNASTAR). Sequence data for recipients of organs from the same donor were aligned using the software

DNASTAR. A sequence was considered to be different from another sequence if there was at least 1 nucleic acid mismatch.

Analysis of the origin of CMV strains. To determine the origin of the CMV strain in CMV-seropositive recipients (i.e., reactivation vs. superinfection), we compared the gB genotype in a seropositive recipient to that in the seronegative counterpart who had received an organ from the same donor. In the case of triplets (donors who had 3 recipients who developed CMV viremia) with 2 seropositive recipients, we compared both seropositive recipients with the only corresponding seronegative recipient. We defined primary CMV infection as the infection that developed in seronegative recipients. Superinfection was defined as a CMV infection in a seropositive recipient when the CMV strain originated from the donor organ. We reasoned that, in this case, both the gB genotype and the sequence of the gB gene should be identical to those of the seronegative counterpart. Reactivation was defined as a CMV infection in a seropositive patient when the strain came from the same recipient. In this case, either the gB genotype or the sequence of the gB gene should differ between the seropositive and the seronegative recipients of organs from the common donor. This last assumption may be limited by the fact that, in the case of transmission of multiple strains from the donor, a seropositive recipient can be superinfected by a strain differing from that transmitted to the paired seronegative recipient.

Statistical analysis. Categorical variables were compared using the χ^2 or Fisher's exact test. Continuous variables were compared using the Mann-Whitney *U* test. All analyses were performed using SPSS software (version 15.0), and $P \leq .05$ was considered to indicate statistical significance.

RESULTS

Study population. A flow diagram of the study is shown in figure 1. Seventy-eight donors and their corresponding 247 recipients were identified. For 57 donors, none or only 1 of the recipients developed CMV viremia and, thus, were excluded. For the remaining 21 donors, at least 2 of their corresponding recipients developed CMV viremia and, thus, were included ($n = 47$ recipients with CMV viremia). The mean age of the donors was 35 years (standard deviation, 18 years). The baseline characteristics of the recipients who developed CMV infection are shown in table 2.

Sixteen of the donors each had 2 recipients who developed CMV infection (pairs). The D/R serostatus of these pairs was as follows: 10 of 16 recipient pairs consisted of 1 seropositive recipient (D^+/R^+) and 1 seronegative recipient (D^+/R^-); for 3 pairs both recipients were seronegative (D^+/R^-), and for 3 pairs both recipients were seropositive (D^+/R^+) (figure 2). Five of the donors each had 3 recipients who developed CMV viremia (triplets). Among the 5 recipient triplets, in 3 instances 2 recipients were seronegative and 1 was seropositive, and in 2 instances 2 recipients were seropositive and 1 was seronegative (figure 3).

Table 2. Baseline characteristics of the transplant recipients included in the study.

Characteristic	Value ($n = 47$)
Age, mean \pm SD, years	48 \pm 13
Sex, male/female	30/17
Organ transplanted	
Kidney	24 (51)
Heart	10 (21)
Lung	6 (13)
Liver	6 (13)
Kidney/pancreas	1 (2)
Pretransplant CMV serostatus	
Seropositive	23 (49)
Seronegative	24 (51)
Induction therapy	
Antilymphocyte therapy ^a	22 (47)
Anti-IL-2 receptor	14 (30)
None	11 (23)
Maintenance immunosuppression	
Prednisone	44 (94)
Tacrolimus	31 (66)
Cyclosporine	16 (34)
MMF	40 (85)
Azathioprine	1 (2)
Sirolimus	3 (6)
Antiviral prophylaxis ^b	
Valganciclovir	3 (6)
Oral ganciclovir	22 (47)
Intravenous ganciclovir	8 (17)
None	19 (40)

NOTE. Data are no. (%) of patients, unless otherwise indicated. CMV, cytomegalovirus; IL, interleukin; MMF, mycophenolate mofetil; SD, standard deviation.

^a Used either as induction therapy or as treatment of delayed graft function.

^b Five patients received sequential therapy with intravenous ganciclovir and either oral ganciclovir or valganciclovir.

CMV gB genotype. Of the 47 recipients who developed CMV viremia, 24 (51%) were infected with genotype gB1, 9 (19%) with gB2, 4 (9%) with gB3, and 10 (21%) with >1 gB genotype (mixed infection). Mixed infections included gB1/gB2 ($n = 3$), gB1/gB3 ($n = 4$), gB1/gB4 ($n = 2$), and gB3/gB4 ($n = 1$). Infection with gB4 alone was not identified. CMV gB genotype was not associated with any particular type of organ transplant ($P = .925$).

Figure 2 shows transmission patterns on the basis of gB genotype analysis for all 16 recipient pairs. For 10 (63%) of 16 recipient pairs, the infecting virus strain was the same in both recipients (i.e., both recipients of an organ from a common donor had infection with the same CMV gB genotype). For the remaining 6 recipient pairs (38%), infection occurred with a different strain despite a common donor. For 5 (83%) of these 6 recipient pairs, at least 1 member of the pair was seropositive for CMV

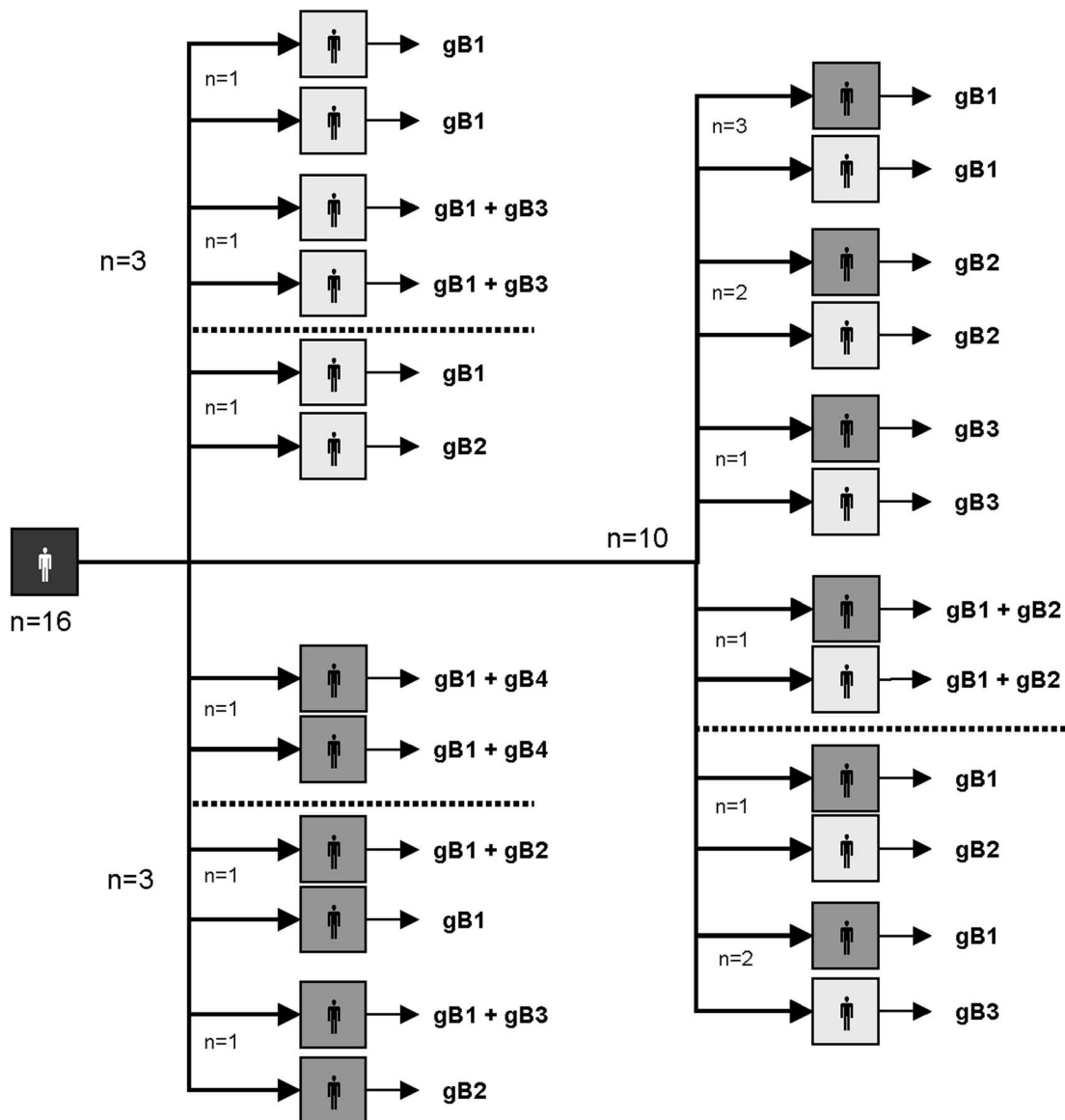


Figure 2. Analysis of the 16 recipient pairs with cytomegalovirus (CMV) infection, according to the pretransplant CMV serostatus of the recipients. The dotted lines separate those recipient pairs infected with concordant CMV glycoprotein B (gB) genotypes from those infected with discordant gB genotypes. The black square denotes CMV-seropositive donors, the dark gray squares denote CMV-seropositive recipients, and the light gray squares denote CMV-seronegative recipients.

before transplant. However, in 1 instance (17%), both of the recipients were seronegative before transplant, and yet each developed an infection with a different CMV strain (gB1 vs. gB2). Interestingly, the gB1-infected recipient then developed a relapse of CMV infection 210 days later, this time with a gB2 genotype. The gB2 genotype and sequence were identical to those of the CMV strain from the matched recipient counterpart. Concordance between genotypes was seen in only 2 (40%) of the 5 recipient triplets (figure 3).

Analysis of CMV origin in seropositive recipients. By analyzing strain relatedness, we determined whether seropositive transplant recipients who developed CMV viremia did so with

the donor strain (superinfection) or via reactivation of their own endogenous strain. This was done by comparing strain similarity between a seropositive recipient and a seronegative recipient of organs from the same donor (see Methods). A total of 17 seropositive recipients were compared with 17 seronegative recipients, as shown in table 3. In 5 (29%) of 17 instances, the gB genotype for the seropositive recipient was different from the gB genotype for the seronegative counterpart. In the remaining 12 instances (71%), the gB genotype was identical for the seropositive and seronegative recipients. In this group, we subsequently attempted to sequence the gB gene to determine the relatedness of strains. Sequencing was successful for 10 (83%) of 12 recipient

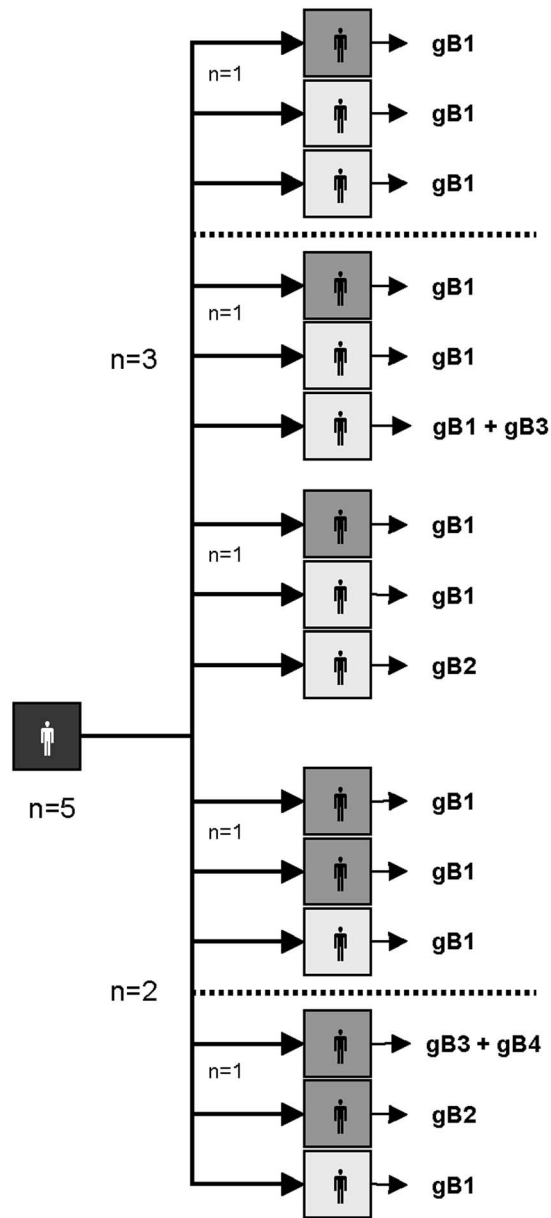


Figure 3. Analysis of the 5 recipient triplets with cytomegalovirus (CMV) infection, according to the pretransplant CMV serostatus of the recipients. See figure 2 for definitions of symbols. gB, glycoprotein B.

pairs. For 3 pairs, the sequence of the gB gene differed between the seropositive and the seronegative patient. For the 7 other pairs, the sequence was identical. Thus, by applying the genotyping and sequencing results to 15 seropositive patients, for 8 (53%) the CMV strain was thought to originate from the recipient (reactivation), and for 7 (47%) the CMV strain was thought to originate from the donor (superinfection).

Occurrence of CMV infection and disease. Clinical outcomes were assessed for all 47 recipients. No clinical data could be obtained for 1 patient, because follow-up was done at another site. Of the remaining 46 patients, 21 (46%) developed symptomatic CMV disease, and the rest had asymptomatic viremia.

Disease type included CMV syndrome (15/21 [71%]) and tissue-invasive disease (6/21 [29%]; all 6 had gastrointestinal disease). No particular gB genotype was associated with the development of CMV disease versus asymptomatic infection ($P = .33$). CMV disease was seen less frequently among patients infected simultaneously with >1 gB genotype (2/10 [20%]) than among patients infected with a single gB genotype (19/36 [53%]), but this difference was not statistically significant ($P = .08$). The only risk factor associated with the development of CMV disease was pretransplant CMV serostatus. The incidence of CMV disease was higher among D^+/R^- patients (16/24 [67%]) than among D^+/R^+ patients (5/22 [23%]) ($P = .003$). Other risk factors, such as the type of organ transplanted or the use of induction therapy, were not associated with the development of CMV disease.

Viral loads. Because the genotyping assay that we used is also quantitative, genotype-specific viral loads measured before the initiation of antiviral therapy were compared between recipients according to the supposed source of the CMV strain. CMV load was higher among patients with primary infection (median, 38,303 copies/mL) than among patients with either superinfection or reactivation ($P = .04$). There was no difference in viral load between seropositive patients with superinfection and those with reactivation (median, 4180 vs. 9495 copies/mL; $P = .54$). The ratio of CMV loads for recipient pairs (the viral load of one recipient divided by the viral load of the other recipient) was not different between pairs infected with the same genotype and pairs infected with different genotypes.

DISCUSSION

In the present study, we assessed donor-to-recipient CMV transmission patterns by comparing CMV genomic variants (gB genotype and gB gene sequence) between seropositive and seronegative recipients of organs from common donors. This is one of the largest studies addressing CMV transmission patterns in organ transplantation. Overall, using a sensitive real-time PCR assay for the detection of CMV genomic variants, we found that CMV transmission demonstrated a complex, dynamic pattern, given that (1) multiple CMV strains can be transmitted from donors to recipients and can be detected simultaneously or sequentially and (2) seropositive recipients can either reactivate their own CMV strain or be superinfected with a strain from a donor (or both). Our data suggest that, among seropositive recipients, approximately half of the infecting CMV strains originate from the organ donor (superinfection), and the other half are endogenous latent virus strains that have reactivated. No differences in virologic or clinical characteristics were found between seropositive patients with superinfection and those with reactivation.

The majority of studies investigating the transmission of CMV from donors to recipients were published ~20 years ago

Table 3. Analysis of the origin of the cytomegalovirus (CMV) strain in 15 seropositive transplant recipients, by comparison of glycoprotein B (gB) genotype and the sequence of the gB gene to those for the seronegative recipient of an organ from the same donor.

Pair no.	gB genotype		gB sequence	Mismatches between sequences, ^a no.	Type of CMV infection in the seropositive recipient ³
	Seropositive recipient	Seronegative recipient			
1	gB1	gB1	Identical	0	Superinfection
2	gB1	gB1	Identical	0	Superinfection
3	gB1	gB1	Identical	0	Superinfection
4	gB1	gB1	Identical	0	Superinfection
5	gB1	gB1	Different	1	Reactivation
6	gB1	gB1	Different	1	Reactivation
7	gB1	gB2	Not applicable ^b	...	Reactivation
8	gB1	gB3	Not applicable ^b	...	Reactivation
9	gB1	gB3	Not applicable ^b	...	Reactivation
10	gB2	gB1	Not applicable ^b	...	Reactivation
11	gB2	gB2	Identical	0	Superinfection
12	gB2	gB2	Identical	0	Superinfection
13	gB3	gB3	Identical	0	Superinfection
14	gB1 + gB2	gB1 + gB2	Different ^c	2	Reactivation
15	gB3 + gB4	gB1	Not applicable ^b	...	Reactivation

NOTE. For 2 recipient pairs (both with gB1 infection in the recipients), results of gB gene sequencing were not available.

^a See Methods for definition.

^b Sequencing was performed only when genotypes were concordant.

^c Sequences of the predominant strain were compared.

[6–10], when the advent of restriction-enzyme analysis of viral DNA allowed the typing of CMV strains. In a study published in 1986 involving 36 pairs of kidney recipients, 4 pairs of recipients with CMV infection were identified [7]. For all 4 recipient pairs, the CMV strain was identical in both recipients by restriction-enzyme analysis, suggesting that the CMV strain was of donor origin. Interestingly, a subsequent study from the same author showed that >1 strain could reactivate in both recipients at different time points [8], an observation in keeping with the results of the present study. Grundy et al. [6] also investigated the concordance of CMV strains in 7 pairs of recipients of kidneys from seropositive donors. The CMV strains were compared on the basis of restriction fragment-length profile. In that study, concordance between strains was high (5/7 pairs), and superinfection with the donor strain was presumed in 6 of 7 seropositive recipients. Several explanations may account for the differences in the probable origin of the CMV strain in these studies compared with the present study. First, the relatively modest sample size in all studies may account for the differences. Second, differences in the type of organ transplanted (previous studies have mostly included kidney transplants) and the antiviral strategy used may also have affected the transmission patterns of CMV. Finally, differences in the sensitivity of the assay techniques used may also partially explain some of the discrepancies among studies. The molecular techniques used in the present study had a greater sensitivity than the techniques used in the previous stud-

ies—hence, the higher incidence of mixed infection with different CMV strains observed here.

Given the relatively high incidence of infection with multiple CMV strains, the main limitation of the present study is the inability to determine the origin of CMV in mixed infections. For example, it is possible that some infections that we have classified as reactivation on the basis of strain differences between the seropositive and seronegative recipients may actually have resulted from 2 different strains being transmitted from the shared donor. In that regard, we observed one instance in which a pair of seronegative recipients had an initial CMV infection with different gB genotypes, but a subsequent relapse of CMV infection in one of the recipients was with a strain identical to the initial strain detected in the other recipient. Transfusion-transmitted CMV represents another potential source of infection in patients who are seronegative before transplant. However, a detailed study of transfusion-transmitted CMV infections in solid-organ transplant recipients who received transfusions of unscreened blood products at our center from 1984 through 1996, when blood products were not universally leukodepleted in Canada, suggests that even then the incidence of transfusion-transmitted CMV infection in this population is extremely low (2.4%) [19]. Transmission via blood transfusion likely has been virtually eliminated since the introduction of universal leukodepletion of blood products in Canada in 2000 [20]. We therefore be-

lieve that CMV transmission from blood had no significant impact on the present study.

Some studies have suggested that gB genotypes may influence the outcome of CMV infection in the immunocompromised host. For example, gB2 was associated with a higher incidence of CMV retinitis in human immunodeficiency virus–infected individuals [15], and infection with gB1 was a risk factor for the development of acute rejection after liver transplant [21]. Other studies have suggested that infection with multiple gB genotypes may have a worse outcome than infection with a single genotype [13, 14]. We did not find any association between gB genotype and the outcome of CMV disease or organ tropism, probably because of the limited sample size in our study. Among patients who were seropositive before transplant, we found no difference in median viral loads between those experiencing superinfection and those experiencing reactivation. However, this analysis was based on viral loads measured in samples used for genotyping analysis, and it is possible that peak viral loads in patients followed up sequentially (particularly in the absence of antiviral treatment) would not differ between these 2 subgroups. The lack of difference in viral load among gB genotypes was also likely the result of the modest sample size in our study.

In conclusion, we have shown that CMV transmission patterns after organ transplant are complex because of the transmission of multiple strains from donor to recipients. However, our data demonstrate that, in D⁺/R⁺ transplant recipients, CMV infections occur as a result of reactivation of endogenous virus or of superinfection with donor virus in similar proportion. In addition, no association was found between gB genotype and the clinical characteristics of the CMV infection.

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