Antibody Maturation and Viremia after Primary Cytomegalovirus Infection, in Immunocompetent Patients and Kidney-Transplant Patients

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To investigate antibody maturation and serum levels of cytomegalovirus (CMV) DNA after primary CMV infection, we studied 51 immunocompetent and 27 kidney-transplant patients. Compared with the immunocompetent patients, the transplant patients had significantly more-prolonged and -variable antibody maturation, clearly longer durations of viremia, and higher levels of CMV DNA; however, antibody maturation continued for >1 year even in immunocompetent patients. Long-term ganciclovir prophylaxis in the transplant patients was associated with either delayed immunoglobulin-G seroconversion, inhibition of antibody maturation (n = 2), or immunoglobulin-class switching (n = 1). In conclusion, antibody maturation continues in immunocompetent patients for a period longer than previously had been thought and is significantly delayed or even inhibited in kidney-transplant patients.

Cytomegalovirus (CMV) infection is one of the most important infectious complications of solid-organ transplantation [1]. CMV employs multiple mechanisms to evade the host’s innate and specific immunity (reviewed in [2]). As a result, the net level of therapeutic immunosuppression is augmented, and the susceptibility to a variety of other opportunistic infections is increased [1]. In particular, CMV-seronegative recipients (R−) of a transplant from a CMV-seropositive donor (D+) are at risk of a severe course of CMV infection [1].

In these transplant patients, antibody maturation, which is determined on the basis of avidity assays, has been found to require ≥12 months after the onset of primary CMV infection [3]—clearly longer than had been anticipated in previous studies of immunocompetent patients [4, 5]. In these latter studies, however, only 5 of the transplant patients and very few of the immunocompetent patients were followed for >1 year after the onset of CMV infection. In addition, none of these studies directly compared immunocompetent versus immunocompromised patients, and the methods used for the evaluation of antibody avidity differed considerably between studies.

To evaluate both virus-specific–antibody maturation after the onset of primary CMV infection and its relation to CMV dissemination in D+/R− kidney-transplant patients, we studied 2 well-defined cohorts—one comprising immunocompetent patients and the other comprising D+/R+ kidney-transplant patients.

Patients, materials, and methods. Between 1998 and 2002, a total of 51 immunocompetent patients with primary CMV infection were identified. These patients had a mononucleosis-like illness, elevated levels of serum transaminase, CMV-specific IgM antibodies, and a significant increase in CMV-specific IgG antibodies. Primary Epstein-Barr virus (EBV) infection was excluded serologically by demonstration of EBV-specific IgG and EBV nuclear antigen–1 antibodies in the absence of EBV-specific IgM antibodies. From these 51 patients, 151 serum samples were collected at a median of 15 weeks (interquartile range [IQR], 6–35 weeks) after the onset of CMV-related symptoms.

In addition, a serum sample from each of 7 healthy donors of bone marrow was included, as a control group; these individuals donated twice during an interval of ≥1.5 years, because, on both occasions, they were both seropositive for CMV-specific IgG and seronegative for CMV-specific IgM infection during the preceding 1.5 years could be excluded for the second serum sample, which consequently was included in this study. In these control-group cases, the interval between the onset of primary CMV infection and the collection of serum samples was arbitrarily set at 18 months.

The mean age of the 58 immunocompetent individuals was 34 years (SD, 14 years). Details on medical history, clinical signs and symptoms, and levels of serum transaminase were obtained by review of medical records, according to the recommendations of the Viennese Institutional Review Board.

A previous prospective study [6] had enrolled 59 D+/R− kidney-transplant patients between 1995 and 2000. Of these 59 patients, only those 27 who could be followed until the first evidence of productive CMV infection and from whom serum
Figure 1. Antibody maturation and presence of cytomegalovirus (CMV) DNA in serum, after the onset of primary CMV infection, in immunocompetent patients (A and C) and kidney-transplant patients (B and D). The dashed lines indicate either the lower limits of high avidity (upper 2 panels) or the detection of CMV DNA (lower 2 panels).

Samples were available were included in the present study; onset of productive CMV infection was defined as the first detection of either CMV DNA in serum (n = 19) or CMV antigen in peripheral-blood leukocytes (n = 8) [6]. The mean age of these 27 patients was 51 years (SD, 14 years). All patients received an immunosuppressive triple therapy; and none received immunoglobulin preparations; rejection episodes were treated uniformly [6]. Of these 27 patients, 19 (70%) received ganciclovir prophylaxis for a mean period of 97 days after transplantation (SD, 32 days). Sequential serum samples (n = 334) were collected from them within a median period of 44 weeks (IQR, 14–84 weeks) after kidney transplantation.

CMV-specific IgM and IgG antibodies were detected by an in-house ELISA, as described elsewhere [7, 8]. EBV capsid-antigen–specific IgM and IgG antibodies were determined by indirect immunofluorescence [8], and EBV nuclear antigen 1–specific antibodies were determined by a commercially available ELISA (DiaSorin). Rheumatoid-factor absorbent (Dade Behring) was used in the IgM ELISAs, to avoid unspecific results, and none of the samples was heat-treated before being tested.

The antibody-avidity assay was based on the widely used technique of elution of virus-specific IgG antibodies from the solid phase of an ELISA, by means of the detergent diethylamine (DEA) (final concentration, 50 mmol/L) (Merck-Schuchardt) [5, 9]. In previously established assays, antibody avidity has been quantified in terms of the avidity index, which is calculated as the ratio of the optical density (OD) with detergent to the OD without detergent. A high index number indicates a high proportion of mature IgG antibodies with high avidity—and, hence, previous CMV infection. Nevertheless, OD measured by ELISA is a 3-parameter logistic function of antibody concentration [10]. As a consequence, testing of serum samples that contain extremes of antibody concentration may cause false-positive results in low-avidity serum samples and false-negative results in high-avidity serum samples [3]. To avoid this shortcoming, we tested each serum sample at 3 dilutions—1:200, 1:1000, and 1:2000. The OD values measured at each dilution were used to fit a 3-parameter logistic curve for serum samples tested with and without DEA. After logarithmic transformation of these 2 curves, we determined the difference in serum dilutions...
Table 1. Comparison of kidney-transplant patients who did or did not receive ganciclovir prophylaxis after transplantation.

<table>
<thead>
<tr>
<th>Category</th>
<th>Ganciclovir prophylaxis (n = 19)</th>
<th>No antiviral prophylaxis (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), years</td>
<td>49 (27–67)</td>
<td>50 (29–71)</td>
<td>.873a</td>
</tr>
<tr>
<td>Female sex, no. of patients (% of total)</td>
<td>4 (21)</td>
<td>3 (38)</td>
<td>.633b</td>
</tr>
<tr>
<td>Second transplantation, no. of patients (% of total)</td>
<td>2 (11)</td>
<td>0</td>
<td>.487b</td>
</tr>
<tr>
<td>Panel-reactive antibodies, median (range), %</td>
<td>0 (0–4)</td>
<td>0 (0–40)</td>
<td>.751a</td>
</tr>
<tr>
<td>HLA-B/DR mismatch, median (range), no. of mismatches</td>
<td>2.0 (0–3)</td>
<td>1.5 (0–4)</td>
<td>.867a</td>
</tr>
<tr>
<td>Graft rejection, no. of patients (% of total)</td>
<td>6 (32)</td>
<td>7 (88)</td>
<td>.013b</td>
</tr>
<tr>
<td>Treatment with anti-lymphocyte globulin, no. of patients (% of total)</td>
<td>3 (16)</td>
<td>4 (50)</td>
<td>.145b</td>
</tr>
<tr>
<td>Virological follow-up after transplantation, median (range), weeks</td>
<td>108 (28–347)</td>
<td>313 (107–426)</td>
<td>.002a</td>
</tr>
<tr>
<td>Productive CMV infection after transplantation, median (range), weeks</td>
<td>22 (6–84)</td>
<td>7 (4–8)</td>
<td>.001a</td>
</tr>
<tr>
<td>Incidence of CMV disease after CMV infection, no. of patients (% of total)</td>
<td>15 (79)</td>
<td>7 (88)</td>
<td>.528b,c</td>
</tr>
</tbody>
</table>

*a* By Mann-Whitney U test.  
*b* By Fisher’s exact test.  
*c* The incidence of CMV disease was similar in the 2 groups, but the severity of the disease was significantly reduced in the group receiving ganciclovir prophylaxis [8].

that was necessary to yield the same OD value when samples were tested with and without DEA; this difference in serum dilution is the avidity factor (AF), a different measure of antibody avidity. An AF \( \geq 10^{-4} \) was considered to be indicative of high antibody avidity. This cutoff value was determined by computation of relative-operating-characteristic curves for immunocompetent patients and allowed the reliable differentiation between CMV infection that occurred \(<6\) months after collection of serum and that which occurred \(>6\) months after the collection of serum. Only samples with a clearly positive result (OD, \(>0.5\)) in the IgG ELISA were used for determination of antibody avidity. Negative OD values obtained after treatment with DEA were arbitrarily assigned a value of 0.0001. CMV DNA was detected by real-time TaqMan polymerase chain reaction (PCR), as described elsewhere [11], by amplification of part of the US17 genomic region of CMV (detection limit, \(\sim 50\) copies/mL).

Comparison of the 2 groups of serum samples was performed by use of the Mann-Whitney U test, for quantitative parameters, and by Fisher’s exact test, for qualitative parameters. In all statistical tests, \(P<.05\) was considered to be statistically significant. All statistical analyses were performed by use of SPSS commercial software (version 11.0; SPSS).

Results. A total of 51 immunocompetent patients with primary CMV infection were included in the present study. None of these patients received antiviral therapy for CMV infection, and all suffered from mononucleosis-like symptoms. Seroconversion to CMV-specific IgG antibodies was observed in all immunocompetent patients \(\leq 4\) weeks after the onset of CMV-related symptoms. After seroconversion, 103 serum samples were collected from these 51 patients. As shown in figure 1A, antibody avidity increased rapidly during the first 3 months after the onset of CMV disease but increased more slowly thereafter, and maximum avidity was attained \(\geq 1\) year after the onset of CMV disease. At \(>18\) months after the onset of CMV disease, AFs were similar in immunocompetent patients and bone-marrow donors and ranged between \(10^{-1.8}\) and \(10^{-2.7}\) (geometric mean, \(10^{-2.8}\); scatter factor, \(10^{0.6}\)).

To evaluate the kinetics of viremia after CMV infection, all 87 serum samples available from 48 immunocompetent patients were tested by a CMV-specific quantitative-PCR assay. CMV DNA could be detected in 37 (43%) of these 87 serum samples, from 27 of these 87 patients, and virus load decreased rapidly after the onset of CMV disease in these 27 patients (figure 1C).

In addition, a total of 27 D+R- kidney-transplant patients were studied. Primary CMV infection was first diagnosed at a median interval of 19 weeks after transplantation (range, 4–84 weeks; IQR, 7–27 weeks). In contrast to what was seen in the immunocompetent patients, as many as 84 weeks (median, 9 weeks) elapsed between the onset of productive CMV infection and the detection of CMV-specific IgG antibodies, and 1 patient had no immunoglobulin-class switching from IgM to IgG antibodies; this patient was followed for \(>2\) years after the onset of CMV infection, and CMV IgM and CMV DNA were present in 7 serum samples collected 7–16 weeks after transplantation. After seroconversion, 85 serum samples were collected from the 26 IgG-seropositive transplant patients. Onset of productive CMV infection was assumed to be indicative of the onset of IgG antibody maturation, because the patients’ immune systems were most likely not stimulated before significant CMV replication occurred [6]. CMV-specific IgG-antibody maturation was clearly prolonged and more variable in the transplant patients than in immunocompetent patients (figure 1). Antibody avidity increased slowly, and, in most patients, maximum
values were not attained until ≥2 years after the onset of productive CMV infection.

To compare the kinetics of viremia after the onset of primary CMV infection in immunocompetent patients versus that in immunosuppressed patients, all 228 serum samples available from the 26 IgG-seropositive transplant patients were also tested by PCR. CMV DNA could be detected in 93 (41%) of these 228 serum samples, from 19 of these 26 transplant patients. The period of viral dissemination was clearly longer, and the levels of CMV DNA levels were higher, in these 26 transplant patients than they were in the immunocompetent patients (maximum period of viral dissemination, 52 vs. 11 weeks; maximum level of CMV DNA, \(9.2 \times 10^5\) vs. \(5.0 \times 10^5\) copies/mL, respectively) (figure 1).

In terms of age, sex, HLA mismatches, and panel-reactive antibodies before transplantation, transplant patients receiving ganciclovir prophylaxis were similar to those not receiving it (table 1). Patients receiving ganciclovir prophylaxis had a lower incidence of graft rejection but did not receive anti-lymphocyte globulin significantly more often. In 2 patients receiving ganciclovir prophylaxis, AFs were as low as \(10^{-7.6}\) and \(10^{-4.8}\) ≥3 years after the onset of productive CMV infection (figure 1B); these 2 patients did not receive anti-lymphocyte globulin. Compared with patients receiving ganciclovir prophylaxis, those not receiving it did not differ in terms of maximum levels of CMV DNA; however, they did differ in terms of the period of viral dissemination (figure 1D).

**Discussion.** The present study is the first to directly compare, in the sera of immunocompetent patients versus those of kidney-transplant patients with primary CMV infection, both long-term antibody maturation and the level of CMV DNA. We found that antibody maturation/avidity continued in immunocompetent patients for a longer period than previously had been acknowledged [4] and that it reached maximum values ≥1 year after the onset of CMV disease. Although differences in methodology require the exercise of caution when studies of antibody avidity are compared, several previous reports have suggested that the period of antibody maturation is longer for infection with CMV than it is for infection with other viruses: whereas 3–5 months elapse between the onset of primary CMV infection and the detection of IgG antibodies with affinity sufficient to differentiate between recent infection and past infection [4], the corresponding interval in the case of rubella-virus infection or EBV infection is only 1–3 months [9, 12]. CMV is known to use multiple mechanisms to elude its host’s immune response [2]; in particular, CMV’s ability to suppress both antigen presentation and polyclonal-antibody production may explain the present study’s observation of a prolonged period of antibody maturation after primary CMV infection.

When compared with immunocompetent patients, immunosuppressed kidney-transplant patients clearly have a longer period of viral dissemination, higher serum levels of CMV DNA, and prolonged or even inhibited CMV-specific IgG-antibody maturation. Previous studies of solid-organ–transplant patients had already observed both delayed IgG-antibody maturation [3] and a correlation between prolonged viremia and delayed acquisition of high-avidity antibodies after primary infection [13]; however, the patient populations studied were considerably smaller than that in the present study and included only solid-organ–transplant patients. The present study of the humoral immune response to primary CMV infection in immunocompetent patients and in kidney-transplant patients further confirms the significant interference that results from immunosuppressive treatment.

Ganciclovir prophylaxis significantly reduces CMV-associated morbidity and mortality in D+/R− transplant patients [6]; nevertheless, previous studies have suggested that ganciclovir can inhibit the immune response to CMV infection [14]. The present study has found that prolonged ganciclovir prophylaxis in kidney-transplant patients is associated with delayed CMV seroconversion [6] and antibody maturation. In addition, in 3 of the 19 transplant patients receiving ganciclovir prophylaxis, either immunoglobulin-class switching from IgM to IgG antibodies was impaired or antibody maturation was inhibited. Although these observations have to be interpreted with caution, because of both (1) the small number of patients studied and (2) differences in the incidence of—and therapy used for—rejection episodes [6], long-term antiviral prophylaxis with ganciclovir may, in some patients, have considerable adverse effects on the immune response to CMV.

In conclusion, antibody maturation in immunocompetent patients continues for a period longer than previously had been acknowledged, and, because of therapeutic immunosuppression, immunity-evasion mechanisms of CMV, and/or long-term ganciclovir prophylaxis, may be delayed in kidney-transplant patients. Antiviral drugs combined with pretreatment immunization may prove beneficial in some patients.

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**References**


