Long-Term Persistence of Immunoglobulin A (IgA) and IgM Antibodies against Human Cytomegalovirus in Solid-Organ Transplant Recipients

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Received 30 November 1998/Returned for modification 15 February 1999/Accepted 23 April 1999

The retrospective analysis of 494 solid-organ transplant recipients revealed that during the follow-up period (mean duration, 3.2 years) 184 (88%) of 209 anti-human cytomegalovirus (HCMV) immunoglobulin A (IgA)-positive patients remained IgA positive, as did 128 (74.85%) of 171 anti-HCMV IgM-positive patients. We conclude that anti-HCMV IgA and IgM testing for management of clinically relevant HCMV infections in solid-organ transplant recipients is dispensable.

Early detection of clinically relevant activity of the human cytomegalovirus (HCMV) in transplant recipients is crucial for adequate establishment of antiviral therapy. Some early reports (3, 12, 15, 16) and some more recent reports (6, 9, 14) have demonstrated that pp65 testing and measurement of viral load by quantitative determination of HCMV DNA are the most adequate laboratory tools for this purpose. However, in many transplantation units HCMV serology is still performed on a routine basis during long-term follow-up after transplantation. The aim of this study was to assess the diagnostic value of this serologic long-term follow up for solid-organ transplant recipients by determining the duration of anti-HCMV immunoglobulin A (IgA) and anti-HCMV IgM seropositivity. Long-term persistence of these antibodies would preclude any crucial diagnostic role for them, because positive tests after conversion cannot be properly interpreted. In this work, we specifically focused on this subject with a comparably large cohort and follow-up periods of up to 6 years.

The database of our diagnostic laboratory was screened for solid-organ transplant recipients whose IgG, IgM, and IgA antibodies against HCMV and pp65 were determined on at least two occasions posttransplantation. The pp65 test was included because, in addition to the main goal of this study as mentioned above, we initially also intended to evaluate the correlation between the time of IgA and IgM conversions and the time of the first positive pp65 test. As an additional inclusion criterion, the interval between the two determinations had to be at least 180 days to ensure that patients with long-term follow-up were selected. From this cohort, we selected subgroups for individual analyses as described below. Antibody testing by enzyme-linked immunosorbent assay was performed with a commercially available test kit (Enzygnost CMV; Dade Behring, Marburg, Germany) according to the manufacturer’s instructions. The plausibility of the cutoff values for IgA and IgM given by the manufacturer was controlled by testing 25 healthy individuals (staff members, aged 18 to 34 years) (see also reference 5). The pp65 antigenemia assay was performed qualitatively with a commercially available indirect immunofluorescence test (CINAkit; Argene Biosoft, Varillies, France). The instructions of the manufacturer were also strictly followed. Primary infections were defined by an anti-HCMV IgG seroconversion resulting in stable IgG positivity (i.e., longer than 180 days). HCMV IgG titers which disappeared at any time after conversion were regarded as a result of passive antibody transfer.

Four hundred ninety-four solid-organ transplant recipients (371 kidney, 83 heart, and 40 liver recipients; 15,141 specimens) were enrolled in this study according to the criteria above (Table 1). Of these, 84 (17.00%) remained completely HCMV antibody negative by all markers used in this study (mean duration of follow-up for this subgroup, 2.57 years). Twenty (4.04%) patients had a primary infection as determined by anti-HCMV IgG seroconversion. In addition to IgG seroconversion, 14 of these primary infections were accompanied by anti-HCMV IgA and IgM seroconversions, four patients seroconverted in IgM only, and one exhibited only IgA. For one patient, no IgM or IgA response could be detected.

Of the remaining 390 patients, 108 (27.69%) exhibited isolated positive IgA results, 25 (6.41%) patients showed isolated positive IgM tests, and 140 (35.89%) patients showed simultaneously positive IgA and IgM results. For the subgroup of patients with positive IgA or IgM results, 171 IgM conversions and 209 IgA conversions were observed. The remaining 11 IgM-positive patients and 53 IgA-positive patients were already positive at the time of entry into the study. The remaining 117 patients had IgG titers without detectable IgM or IgA antibodies at any time.

A preliminary analysis of the interval between seroconversions and positive pp65 results revealed an approximately symmetric distribution of seroconversions for both IgA and IgM around the first positive pp65 result (data not shown). A fine analysis of the data (data not shown) showed that this was mostly due to the retrospective design of this study, because consequent parallel testing of all parameters considered here was performed only during the first 2 months after transplantation; for most patients during ambulant follow-up, pp65 testing was done only sporadically. Due to this lack of data, we were not able to directly evaluate the role of the pp65 antigenemia test for long-term follow-up in our patient cohort.

In order to quantify the persistence of IgA seropositivity, we selected the patients with detectable IgA seroconversions and correlated the duration of IgA seropositivity after seroconversion with the duration of subsequent follow-up. In Fig. 1, the two parameters are compared, showing that in the majority of cases IgA results remained positive for as long as the
patients were monitored. Only for 25 (11.96%) of 209 IgA seroconverters did IgA antibodies fall below the detection limit. In addition, the frequency of reversions was not correlated with the duration of positivity (data not shown). The mean duration of follow-up was 1.86 years in this group, including 22 cases with follow-up periods from 5 to 6.13 years.

If the same approach was applied to IgM (Fig. 2), it turned out that anti-HCMV IgM also persisted in the respective patients. Of 171 evaluable IgM seroconverters, only for 43 (25.14%) could an IgM reversion be demonstrated. As was the case with anti-HCMV IgA antibodies, the frequency of reversions was not correlated with the duration of positivity (data not shown). The mean duration of follow-up was 1.94 years in this group, including 21 cases with follow-up periods from 5 to 5.93 years.

Results from our immunocompetent control group showed that 6 of 25 (24%) had anti-HCMV IgG antibodies; no IgA or IgM antibodies against HCMV could be detected in this group (see also reference 5).

Publications specifically providing data concerning the duration of anti-HCMV IgA positivity in solid-organ transplant recipients are rare; some have reported shorter times of IgA positivity, which could in part be ascribed to documented reversions (7, 17); however, in two studies by Sarov et al. (10, 11) over 90% of the IgA-positive kidney recipients did not revert for as long as they were monitored (numbers of patients were 12 and 10 and durations of follow-up were, maximum, 66 and 60 weeks, for references 10 and 11, respectively). Under these circumstances, it could not be firmly determined whether IgA reversions would have occurred if patients had been monitored longer. In this paper, we provide evidence (by the data for 494 patients monitored for up to 6 years) that persistence of anti-HCMV IgA in transplant recipients is virtually unlimited and not correlated with observation periods. Given the high prevalence of anti-HCMV IgA in solid-organ transplant recipients (42.3% in this study), anti-HCMV-testing is justified only if seroconversion is an early and sensitive indicator for clinically relevant HCMV activity. A more recent study (8) comparing a broad panel of serological tests with pp65 antigenemia and PCR in detail revealed that IgA testing was of only minor importance under this aspect.

Although there have been some considerable efforts to improve sensitivity of anti-HCMV IgM tests by combining recombinant antigens with immunoblotting techniques (1, 2, 4), recent studies (3, 6, 9, 14) have shown that pp65 antigenemia testing and quantification of HCMV DNA are by far superior to any of the evaluated serological tests for detection and monitoring of clinically relevant HCMV disease. Follow-up periods in these studies were generally shorter than 1 year; however, our long-term data support the notion that anti-HCMV IgM testing is of questionable diagnostic value during long-term follow-up, too.

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<tr>
<th>Conversion</th>
<th>No. of recipients with result:</th>
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<tbody>
<tr>
<td></td>
<td>HCMV negative</td>
</tr>
<tr>
<td>None (IgA or IgM)</td>
<td>84</td>
</tr>
<tr>
<td>IgA</td>
<td>0</td>
</tr>
<tr>
<td>IgM</td>
<td>0</td>
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<tr>
<td>IgA-IgM</td>
<td>0</td>
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<tr>
<td>Total</td>
<td>84</td>
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Combined testing for anti-HCMV IgA or IgM may be of some use for retrospective confirmation of HCMV reactivations diagnosed with other tools in clinical trials focusing on the benefit of prophylactic or preemptive antiviral regimens. In summary, however, we conclude that routine IgA and IgM testing of solid-organ transplant recipients is of only questionable value for management of clinically relevant HCMV reactivations in solid-organ transplant recipients. Our serological data and the literature (see above and references 12 and 13) strongly suggest justification for concentrating laboratory resources on more reliable test systems like the pp65 antigenemia test or methods for the quantitative determination of HCMV DNA.

REFERENCES


