Humoral Immune Response to Human Cytomegalovirus DNA Polymerase

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In this work, we show that antibodies to baculovirus recombinant DNA polymerase of human cytomegalovirus are present in human sera during natural infection. Specific antibody was found to be of the immunoglobulin G class, produced at low titers only for a short period of time at the beginning of acute infection and not detectable in sera from patients with a convalescent or a latent phase of infection. These results were compared with those obtained with procaryotically expressed p52, the polymerase accessory protein.

Human cytomegalovirus (HCMV) is an important pathogen for the humans all over the world. While specific immune response does not offer protection against infection, it does influence HCMV-associated disease by either inhibiting it completely or attenuating its severity (for review, see references 3, 4, 9, and 12).

While much information on the immunogenic potential of HCMV structural proteins is available, little about the immunogenicity of nonstructural proteins is known (for review, see reference 5). Among nonstructural proteins of particular interest is the 140- to 145-kDa DNA polymerase (pol 140) encoded by UL54 (1) and recently expressed in baculovirus (2). However, nothing about its potential immunogenicity is known. On the contrary, the 52- to 55-kDa polymerase accessory protein (p52) encoded by UL44 has been shown to be one of the most immunogenic HCMV proteins (5, 6, 10).

This work was designed to determine whether HCMV DNA polymerase can trigger a specific antibody response in vivo, which antibody class is produced, and how the specific antibody response evolves during natural infection compared with the antibody response to the 52- to 55-kDa polymerase-associated protein.

The antigens used in this study were partially purified extract from SF9 cells infected with the recombinant baculovirus and crude extracts of Escherichia coli infected with G2 and D1 recombinant lambda gt11 (Fig. 1).

For conventional serology, an enzyme immunoassay (EIA) for immunoglobulin M (IgM) and IgG detection was performed with two commercial kits. For IgG detection, an indirect HCMV EIA was used (M. A. Bioproducts, Walkersville, Md.). In this test, three calibration serum samples were used, and negative sera were considered to be those giving an EIA optical density (OD) value below 0.198, those with a low titer were in the range 0.198 to 0.359, those with a medium titer were between 0.360 and 1.239, and all EIA OD values of > 1.240 were considered to be high titers. For IgM detection, an antibody capture EIA was used (Technogenetics, Hamburg, Germany). In this case, a positive serum sample and a negative serum sample were used as controls. IgM-positive sera were those with an EIA OD of > 0.210 (Fig. 2).

When extracts of SF9 cells infected with the recombinant baculovirus were tested with human sera from patients with acute HCMV infection, at least four bands with molecular masses of 145, 69, 58, and 35 kDa were visible. Only the band at 140 to 145 kDa (pol 140) was considered HCMV specific because (i) previous studies have shown that only a protein with a molecular mass of 140 kDa was recognized by a synthetic peptide-induced antibody directed to the N terminus of the DNA polymerase sequence in extracts from SF9 cells infected with the recombinant baculovirus (2), (ii) the other bands are also present in wild-type baculovirus-infected cell extracts, and (iii) antibodies present in HCMV-negative sera often react in different combinations with the other bands in infected cell extracts (data not shown).

To determine the frequency of serum positivity to HCMV DNA polymerase, 154 serum samples from transplant patients, pregnant women, and patients with AIDS were divided into five subgroups depending on the anti-HCMV antibody titers (Fig. 1) and were tested for the presence of antibodies specific for pol 140 as follows. Group 1 was composed of 16 HCMV-negative serum samples; groups 2 and 3 were composed of IgM-negative, IgG-positive sera with low levels and medium to high levels of IgG, respectively. Group 4 was composed of 65 IgM-positive serum samples with medium to high levels of IgG, and group 5 contained 12 IgM-positive, IgG-negative serum samples. The last subgroup of sera was selected from more than 5,000 serum samples from our collection and represents sera from patients during the very early phase of primary HCMV infection. (In fact, the seroconversion for HCMV-specific IgG was detected 4 to 8 days later.)

Sera were routinely tested by immunoblotting (7) at a 1:10 dilution for pol 140 and a 1:50 dilution for p52 and pL50. Sera giving a positive reaction were tested at higher dilutions; those negative at a 1:10 dilution were further tested at a 1:5 dilution, the reaction being amplified by the biotin-streptavidin method. All sera that were positive for IgM were tested for the presence of rheumatoid factor by latex agglutination (Rheuma Wellcotest; Wellcome Research Laboratories, Beckenham, England). Only rheumatoid factor-negative sera were included in this study. Sera were routinely tested with proteins from extracts of SF9 cells infected with wild-type baculovirus and of E. coli cells infected with wild-type lambda gt11.

The DNA-polymerase antigen was prepared as described...
by Ertl et al. (2a). Briefly, lysates of SF9 cells infected with recombinant or wild-type baculovirus were sonicated in the presence of dithiothreitol and centrifuged at 14,000 × g; the resulting pellet was resonicated in the presence of a high salt concentration and Nonidet P-40. The insoluble material collected by centrifugation was considered a partially purified preparation of recombinant HCMV DNA polymerase (2). Proteins present in this material were separated by polyacrylamide gel electrophoresis, Western blotted, and used to test human sera. One microgram of partially purified pol 140 was used for each serum sample. Recombinant p150 and p52 were prepared as described previously (10). Recombinant p150 corresponds to the D1 fusion protein containing the last 25 amino acids at the carboxy terminus of phosphoprotein p150 (UL32), which is the major structural antigen, while recombinant p52 corresponds to the G2 fusion protein corresponding to amino acids 1071 to 1301 of the 52-kDa nonstructural DNA binding protein (UL44).

As shown in Fig. 1, HCMV-negative sera as well as sera with a low level of IgG specific for CMV did not show any reactivity to pol 140, while 20% of serum samples with a medium to high level of IgG specific for HCMV reacted with pol 140. Furthermore, >40% of the IgM-positive serum samples with high levels of IgG reacted with pol 140. In a small group of IgM-positive, IgG-negative serum samples, the percentage of reactivity to pol 140 was the highest found in our study (58.3%). Specific antibody titers observed in sera from this group were higher than those detected in sera from the other groups (data not shown). All of the sera were tested for their reactivity to another nonstructural antigen (p52) and a structural antigen (p150). As shown in Fig. 1, antibody specific for p52 shows a distribution similar to that of antibodies specific for pol 140, but the percentages of reactivity as well as the titers of specific antibodies (data not shown) were much higher. On the contrary, the percentages of antibodies specific for p150 were uniformly distributed in the three IgG-positive serum samples irrespective of their IgG titers. As expected from previous data (6), the percentage of reactivity was low in IgG-negative, IgM-positive sera.

To test whether antibodies to pol 140 are produced exclusively during primary infection, 84 serum samples were used. Among them, 52 were from transplanted patients undergoing a primary HCMV infection. These patients were seronegative at the time of transplantation and became seropositive sometime later. The other 32 specimens were from transplant patients undergoing a nonprimary HCMV infection. These patients were already seropositive at the time of transplantation, and their EIA (IgG) values had increased at least 1.5 times. As shown in Table 1, very similar percentages of reactivity to pol 140 were detected in both groups. As was also expected, a strong IgG reactivity to p52 and p150 was detected in both groups.

To determine the antibody class involved in the reaction to pol 140, 10 pol 140-positive serum samples were tested separately for detection of IgG and IgM activity. Nine out of 10 serum samples showed only IgG reacting with pol 140. One serum sample which was judged positive with a total anti-Ig conjugate gave a negative result when tested separately with an anti-IgG or IgM conjugate (data not shown).

**Table 1. Number of serum samples giving positive reactions with pol 140, p52, and p150**

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<tr>
<th>Type of infection (no. of subjects)</th>
<th>No. (%) of serum samples giving a positive reaction with:</th>
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<tr>
<td>Primary (52)</td>
<td>pol 140 28 (53.8) p52 40 (76.9) p150 30 (57.7)</td>
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<tr>
<td>Nonprimary (32)</td>
<td>pol 140 16 (50.0) p52 28 (87.5) p150 32 (100)</td>
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FIG. 1. Percentage of reactivity to DNA polymerase pol 140 (p 140), p52 (p 52), and p150 (p 150) of antibodies present in serum groups 1 to 5 (see text for details).

FIG. 2. Evolution of the humoral immune response to DNA polymerase and the polymerase accessory protein (p52) in a group of six transplant patients (panels 1 to 6) undergoing a primary HCMV infection during the first 6 months after transplantation. Four patients had had a kidney transplant, one had had a liver transplant, and one had had a heart transplant. All patients had received combination immunosuppressive therapy and had not received any HCMV-specific chemotherapy or immunoprophylaxis. Time zero is the time of the last seronegative serum sample detected by IgG and IgM EIA. Titers of antibodies are expressed as logs of the reciprocal of the serum dilution. -- -- --, antibodies specific for pol 140; ---, antibodies specific for p52.
Finally, a group of six transplant patients undergoing an active HCMV primary infection during the first 2 to 6 months after transplantation (Fig. 2) were checked for the appearance of and progressive changes in IgG specific for p140 during the first 3 months after the last seronegative serum sample (detected by EIA for both IgG and IgM) in comparison with the appearance and evolution of antibodies specific for p52. The results obtained are shown in Fig. 2. In five out of six patients, the appearance of antibodies to pol 140 coincided (four patients) or occurred 1 week later (one patient) than the reactivity to p52. In one patient (patient 6), the reactivity to pol 140 was not detected even when the titer of antibody specific for p52 was very high. The response to pol 140 was more transient than that specific for p52, and in four out of five patients antibodies specific for pol 140 had already disappeared when those specific for p52 were still at high levels (and the patients were still viruic; data not shown).

In conclusion, our results indicate that DNA polymerase is immunogenic during natural infection. Antibodies specific for DNA polymerase are produced during the acute phase of infection (both primary and nonprimary) in at least 58% of the infected subjects. The most likely explanation for the negative results obtained in 42% of the subjects is that the level of specific antibodies was too low or antibodies were present for a too short a period to be detected under our experimental conditions. This is supported by the fact that titers of antibodies specific for the DNA polymerase range from 1/5 to 1/40 as determined by immunoblotting titration, while titers of antibodies specific for other nonstructural and structural antigens reach 1/2,000 and 1/10,000, respectively, when tested by immunoblotting (unpublished results). Our results also indicate that specific antibodies are mainly produced at the beginning of infection and are detectable in the blood for 4 to 8 weeks, which is a much shorter time than the persistence of antibodies to other viral proteins. We cannot exclude the possibility that antibody binding to conformational epitopes of HCMV DNA polymerase not present in the denatured protein on sodium dodecyl sulfate-polyacrylamide gel electrophoresis could be present in human sera and not detected in our study. However, the few immunoprecipitation experiments we have done do not support this possibility. In the same patients, immune reactivities in response to p52 and p150 were studied, and the results obtained are in agreement with previously published reports (6, 7, 10). In particular, the distribution of antibodies specific for p52 was similar to that of antibodies specific for pol 140. However, the humoral immunity to p52 was much greater and lasted longer than that to pol 140. On the contrary, the distribution of the immune reactivity to p150 differed, immune reactivity to p150 being almost uniformly distributed among IgG-positive sera, irrespective of the titer of IgG specific for HCMV. A low percentage of reactivity to p150 was found in IgG-negative, IgM-positive sera. This result was expected because it is known that antibodies specific for p150 become detectable during primary HCMV infection later than antibodies to other viral proteins (8). The antibody class reacting with pol 140 was found to be IgG, but we cannot exclude that IgM is produced at very low levels and/or for such a short period of time as to be undetectable under our experimental conditions.

In conclusion, this paper represents the first study of the antibody response to a recombinant early HCMV protein and the second study of the immune response to an individual early HCMV protein—the first being a study of a 72-kDa nuclear protein (p72) which was affinity purified from infected cell extracts and shown to induce an immune response comparable to that obtained with virion antigen (11).

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REFERENCES