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Humoral Immune Response against Human Cytomegalovirus (HCMV)-Specific Proteins after HCMV Infection in Lung Transplantation as Detected with Recombinant and Naturally Occurring Proteins

J. VAN ZANTEN,[1] M. C. HARMSEN,[1] M. VAN DER GIESSEN,[1] W. VAN DER BIJ,[2] J. PROP,[3] L. DE LEIJ,[1] AND T. H. THE[1]*

Department of Clinical Immunology,[1] Department of Pulmonary Diseases,[2] and Department of Cardiopulmonary Surgery,[3] University Hospital Groningen, 9713 EZ Groningen, The Netherlands

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The humoral immune response to four intracellularly located cytomegalovirus (CMV) proteins was studied in 15 lung transplant recipients experiencing active CMV infections. Five patients had primary infections, and 10 had secondary infections. Antibodies of the immunoglobulin M (IgM) and IgG classes were measured in an enzyme-linked immunosorbent assay (ELISA) system in which procaryotically expressed recombinant proteins were used as a substrate and also in a monoclonal antibody-based capture ELISA which uses naturally occurring proteins as a substrate. The proteins investigated were the lower matrix protein pp65 (ppUL83), the major DNA-binding protein p52 (ppUL44), and the two immediate early proteins IE1 and IE2 (different splicing products of UL123). Higher levels of antibodies were found to pp65 and especially to p52 than to the immediate early antigens. Antibody levels detected in the recombinant protein-based ELISAs were generally lower than antibody responses detected with the matching antigen capture ELISA. Moreover, some patients appeared to have antibodies mainly to epitopes present on naturally occurring proteins. The antibody responses detected in both assays were related to the viral load during infection as assessed by the CMV antigenemia test, which is a quantitative marker for CMV load. It was found that although epitopes on naturally occurring proteins induce higher antibody responses and responses in more patients, antibodies directed to epitopes present on the recombinant proteins were inversely related to the viral load during a CMV infection. Therefore, antibodies to epitopes on the recombinant proteins might be more clinically relevant in this group of lung transplant recipients.

Cytomegalovirus (CMV) is a common pathogen in humans. Although infection is not associated with clinical symptoms in most cases, active CMV infection can be a major threat in newborns and immunocompromised individuals. For example, in lung transplant recipients, a high incidence of severe CMV infections is seen as possibly due to risk factors such as donor-recipient HLA mismatching and heavy quadruple immunosuppression.

To investigate humoral immune responses to CMV, different techniques like Western blotting (immunoblotting) (3, 4, 10) and various enzyme-linked immunosorbent assays (ELISAs) have been used (2, 14). The mixtures of antigens used as substrates in these various assays were derived from CMV virions or from CMV-infected fibroblasts and may have been prepared by different procedures, thereby possibly leading to different findings and conclusions. More recently, single recombinant CMV antigens expressed as fusion proteins were used as substrates (7, 8). In this way, antibody responses to a defined part of a certain CMV protein can be studied. A disadvantage in this approach is the fact that only a part of an antigen is used and reactivity against the non-CMV part of the fusion protein, such as β-galactosidase, may also be seen.

The aim of the present investigation was to study the kinetics of the humoral response directed to epitopes present on recombinant proteins and to epitopes present on naturally occurring CMV-specific proteins. Four complete recombinant *Escherichia coli*-expressed nonfusion proteins were used for antigen: the lower matrix protein pp65, the major DNA-binding protein p52, and the immediate early proteins IE1 (72 kDa) and IE2 (86 kDa). The first proteins were chosen for their relative abundance and expected antigenicity (16). The immediate early proteins were chosen because they are the first CMV antigens produced in an infected cell and because they have a regulatory function in the further development of the virus in the cell. Therefore, an immune response to these proteins might influence the further development of a CMV infection. In fact, cellular immunity to IE1 has been investigated in detail in humans as well as in mice (1, 6) and has been proven to be important in the viral control of the host. The humoral response to IE1 is still unknown, however.

A possible drawback in using recombinant proteins expressed in *E. coli* is that these antigens have not undergone posttranslational modifications and appear to have improper tertiary folding. Since antibodies can be missed in an ELISA when recombinant proteins are used as antigens, we also investigated antibody responses of patients against conformational epitopes present in CMV-infected fibroblasts. Here we report an investigation into both types of responses to p52, pp65, and IE2 using an antigen capture ELISA to detect antibodies to conformational epitopes and a recombinant protein-based ELISA to detect antibodies to linear nonglycosylated and nonphosphorylated epitopes. To our knowledge, this is the first study in which antibody responses to complete CMV-specific proteins have been investigated. Moreover, the

* Corresponding author. Mailing address: Dept. of Clinical Immunology, University Hospital Groningen, Oostersingel 59, 9713 EZ Groningen, The Netherlands. Fax: +31 50 613474.

difference in responses to conformational and linear epitopes was related to the viral load of the patients as detected by the CMV antigenemia test (12, 13).

MATERIALS AND METHODS

Patients. Sera from 15 lung transplant recipients were evaluated retrospectively. Five patients had primary CMV infections (the donor was CMV seropositive and the recipient was seronegative), and 10 CMV-seropositive recipients had secondary CMV infections (6 of 10 received a lung from a CMV-seronegative donor). An active CMV infection was defined by detection of CMV antigen-positive leukocytes in the antigenemia test and/or as a seroconversion or a significant rise of immunoglobulin G (IgG) antibodies determined in the multispecific ELISA (see below). The onset of an active CMV infection was defined as the time point at which one of the above tests was found positive. Serum samples were taken once a week.

In the patients with primary CMV infections, a positive antigenemia test was always found before an IgM seroconversion as assessed in the multispecific ELISA. The maximum number of CMV antigen-positive cells in the CMV antigenemia test varied between 4 and 343, the median being 50 per 50,000 leukocytes. All patients with secondary CMV infections had significant increases in IgG antibodies, and 9 of 10 had positive antigenemia tests. Here, the maximum number of positive cells ranged between 0 and 156, with a median of 4 per 50,000 leukocytes.

The immunosuppressive regimen consisted of a combination of cyclosporin (level, 400 μg/liter until day 5 and declining from 300 to 150 μg/liter at day 22), azathioprine (1.5 to 3 mg/kg/day), prednisolone (125 mg three times in the first 24 h and a minimum of 15 mg/day for the next 3 months), and rabbit antithymocyte globulin (3 mg/kg given in the first days after transplantation). CMV-seronegative recipients who received a CMV-seropositive graft were given hyperimmune anti-CMV globulin (Cytotect; Biotest Diagnostics, Dreieich, Germany) at 2 ml/kg on day 0 and at 1 ml/kg on days 2, 7, 14, and 28 posttransplantation as a prophylactic treatment.

Antigenemia test. The detection of CMV antigen-positive blood leukocytes was carried out as described by Van der Bij et al. (13). In short, peripheral blood leukocytes were isolated by dextran sedimentation and cytocentrifuged onto glass slides. These slides were air dried, fixed with water-free acetone, and stained with a mixture of monoclonal antibodies C10 and C11 (raised in our laboratory and commercially available from Biotest Diagnostics) by an indirect immunoperoxidase technique. The test was carried out in duplicate. The total number of CMV antigen-positive cells was scored and expressed per 50,000 leukocytes screened. This is the approximate number of cells present on one slide. If only one CMV-positive cell was found, the result is given as <1 per 50,000.

Multispecific ELISA. Total CMV-specific antibody was determined in a quantitative ELISA as described by Van der Giessen et al. (14). Briefly, wells of microtiter plates were coated with alkaline glycine-extracted CMV antigens obtained from human fibroblasts during a late stage of infection. Serum samples were added in serial twofold dilutions starting with 1/100, and bound CMV-specific antibody was detected with a peroxidase-labelled second antibody against human immunoglobulins. Sera were also tested on an extract of mock-infected fibroblasts. The amount of antibody present in the patient serum was calculated relative to that of a standard serum which was included in each plate and expressed as a percentage of the antibody concentration in a standard, which was set at 100 U. In patients with secondary CMV infections, the onset of an antibody response was defined as the time point when the amount of antibody in a given serum sample was 1.5 times as high as that in the previous sample and/or the pretransplantation serum.

Procaryotic expression and purification of proteins. The production of the constructs will be described elsewhere. In short, for procaryotic expression and purification of histidine-tagged proteins, *E. coli* M15 containing the plasmid pREP4 was used. This plasmid carries the gene for neomycin phosphotransferase conferring kanamycin resistance upon *E. coli* cells and the *lacI* gene encoding the *lac* repressor. After transformation of *E. coli* M15(pREP4) with expression constructs containing the pp65, p52, IE1, or IE2 cDNA with a 6-histidine-residue cassette engineered at the 3′ or 5′ terminus, cells were selected on Luria-Bertani plates and grown in Luria-Bertani medium supplemented with 25 μg of kanamycin and 100 μg of ampicillin per ml. Protein synthesis was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) to an exponentially growing *E. coli* culture. After 3 h of induction, cells were harvested and lysed by resuspension of the bacterial pellet in buffer A, which contained 6 M guanidine hydrochloride and 20 mM Tris-HCl (pH 8.0), and then a centrifugation step at 10,000 × *g* for 10 min. Histidine-tagged proteins were purified via metal chelate affinity chromatography using Ni^{2+} nitrilotriacetic acid agarose (Qiagen, Hilden, Germany). Briefly, 10 ml of cell lysate in buffer A was passed through a 1-ml column of Ni^{2+} nitrilotriacetic acid agarose that had been preequilibrated in buffer A. The column was then washed with 30 ml of buffer B (8 M urea, 20 mM Tris-HCl, 20 mM imidazole [pH 8.0]), and the protein was eluted with buffer C (8 M urea, 20 mM Tris-HCl, 100 mM imidazole [pH 8.0]) in fractions of 1 ml. Protein-containing fractions were identified by measuring the optical density at 280 nm and analyzed further by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by either amido black staining or Western blotting. The four recombinant proteins were used directly without further purification steps for coating ELISA plates.

Recombinant protein-based ELISA. Proteins in elution buffer C (see above) were diluted in 0.1 M Na_2CO_3 [pH 9.6] and incubated in microtiter plates (Greiner, Nürtingen, Germany) at 100 μl per well for at least 48 h at 4°C. The optimal concentration for binding was determined for each protein and appeared to range from 0.6 to 2.0 μg/ml. Proteins were coated in the presence of urea as described by Tijssen (11).

The ELISA was done as follows: twofold dilutions of serum, starting from 1/100, were made in incubation buffer (10 mM Tris-HCl [pH 8.0], 0.3 M NaCl, 1% bovine serum albumin, 0.05% Tween 20), and 100 μl was added per well. Incubation periods were 45 min at 37°C, after which the plates were washed with washing buffer (10 mM Tris-HCl [pH 8.0], 0.15 M NaCl, 0.05% Tween 20) with a Titertek ELISA wash apparatus (ICN, Costa Mesa, Calif.). Bound antibodies were detected with horseradish peroxidase-labelled goat anti-human IgG (de Beer Medicals, Hilvarenbeek, The Netherlands) or IgM (Pasteur, Paris, France) diluted 1/2,000 or 1/1,000, respectively.

A reference serum obtained from a patient with a proven anti-CMV titer was included in each assay. The amount of antibody present in a serum sample was expressed as a percentage of this reference serum. Five lung transplant recipients without active CMV infections and five healthy CMV-seronegative individuals were tested with the ELISAs to determine the background level. It appeared to be lower than 1% for both IgM and IgG. Therefore, samples containing more than 1% IgM or IgG antibodies were considered positive. An increase in antibodies was considered significant if the amount of antibody in a given serum sample was two times as high as that found in the previous sample.

Antigen capture ELISA. Levels of IgG antibody directed to single CMV antigens were determined by a modified monoclonal antibody-based antigen capture ELISA as described by Van der Voort et al. (15). Microtiter plates (Labstar; Costar, Cambridge, Mass.) were precoated with a polyclonal antiserum against mouse immunoglobulins (3.2 μg/ml; Jackson, West Grove, Pa.), after which one of the anti-CMV mouse monoclonal antibodies was added. Subsequently, antigen present in a detergent extract of CMV-infected cells in the late stage of infection was allowed to bind to the monoclonal antibody. Anti-CMV antibodies in human sera bound to these CMV proteins were detected by peroxidase-labelled second antibody against human immunoglobulins. The amount of antibody present in the patient serum was expressed as described above. Samples containing more than 1% IgG were considered positive. Three different monoclonal antibodies were used: C10, directed against the lower-matrix protein pp65 (5); CCH2, directed to the major DNA-binding protein p52 (DAKO, Glostrup, Denmark); and IE05-9, which reacts specifically with the IE2 protein which has a molecular weight of 86 kDa. This monoclonal antibody was made in our laboratory by immunization with the recombinant IE2 protein as expressed in *E. coli*. Specificity was demonstrated by immunofluorescence staining on CMV-infected fibroblasts and by peroxidase staining on insect cells expressing the recombinant baculovirus IE2 protein. Moreover, in an immunoblot assay using an extract of CMV-infected fibroblasts or an extract of insect cells positive for the IE2 protein, a staining was seen with a protein having a molecular size of 86 kDa. This monoclonal antibody does not recognize the 72-kDa IE1 protein. No cross-reactivity was seen with other herpesviruses.

Antibody response factor. To compare the antibody responses to the four proteins, the antibody response factor, which is a reflection of the magnitude of the antibody response against a particular protein during a certain period, was calculated. This was done as follows. The level of antibodies against a certain protein before diagnosis of active CMV infection (i.e., before antigenemia) was subtracted from that found in a particular serum sample after diagnosis of CMV, and the difference was divided by the time in weeks that elapsed between the sampling of both sera. This was done for all four recombinant proteins and for all patient serum samples. Per patient, the highest factor per recombinant protein was used for the comparison between antibody responses.

Statistical analysis. Differences between the groups with high and low viral loads and the magnitude of the antibody responses were evaluated by the Wilcoxon test.

RESULTS

Primary CMV infection. IgM antibody levels were found in the patient sera predominantly 3 to 4 weeks after the first positive antigenemia test, but the time ranged between 0.5 to 11 weeks (Fig. 1). When patients developed antibodies to one or more of the recombinant proteins, these antibodies appeared simultaneously with those detected in the multispecific ELISA. In time, all patients (five of five) developed anti-p52, three of five developed anti-pp65, two of five developed anti-IE1, and two of five developed anti-IE2 IgM antibodies. Antibodies specific for all four recombinant proteins could be detected in only two patients, and two patients produced an-

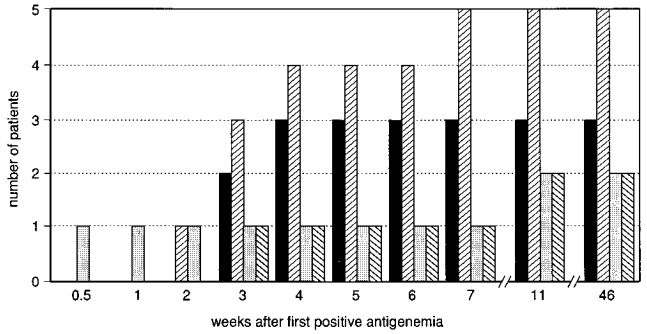

FIG. 1. Appearance of the IgM antibodies during a primary CMV infection in weeks after the first positive antigenemia test. The numbers of patients with responses to the CMV recombinant proteins pp65 (■), p52 (▨), IE1 (▩), and IE2 (▧) were determined.

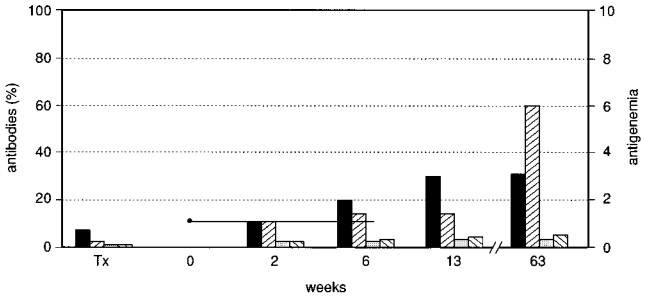

FIG. 3. An example of antibody responses of a patient to pp65 (■), p52 (▨), IE1 (▩), and IE2 (▧) together with the course of the antigenemia test (●——) given in weeks after the first positive antigenemia. Tx, day of transplantation.

tibodies to p52 alone. The highest IgM antibody levels found appeared to be directed against p52 and pp65.

With respect to the development of IgG antibodies, all five patients showed an IgG response in the standard multispecific ELISA. However, in the four recombinant protein-based ELISAs, anti-pp65 antibodies were seen in two of five patients, anti-p52 antibodies were seen in two of five patients, and anti-IE2 and anti-IE1 antibodies were each seen in one of five patients. Thus, in this group with primary infections, few patients had IgG antibodies to the recombinant proteins. These antibodies appeared at the same time as the antibodies detected in the multispecific ELISA, i.e., 3 to 16 weeks after the first positive antigenemia test. Two patients did not show IgG reactivity to any of the four proteins, and another patient had a low antibody level of 3% to p52 only. In one patient, high levels of IgG and IgM antibodies directed to pp65 (>20%) and IE2 (>10%) were found at the same time. This was the patient with the lowest number of CMV-positive cells, i.e., a maximum of 4 of 50,000 leukocytes.

Secondary CMV infection. In general, IgG antibodies to the four different recombinant proteins appeared at the same time as the IgG antibody rise detected in the multispecific ELISA. In some cases, one antibody specificity was detected later than the others, but if so, this differed with each patient. In Fig. 2, the kinetics of appearance of the four antibody specificities are shown. One patient had no positive antigenemia test and was omitted from the results shown in this figure. This patient had a significant level of antibodies to all four recombinant proteins. Thus, all patients (10 of 10) had antibody responses to p52, 6 of 10 had responses to pp65, 5 of 10 had moderate responses to IE1, and 6 of 10 had responses to IE2. In Fig. 3, an example is shown of the antibody responses to the different proteins found in a patient with a secondary infection.

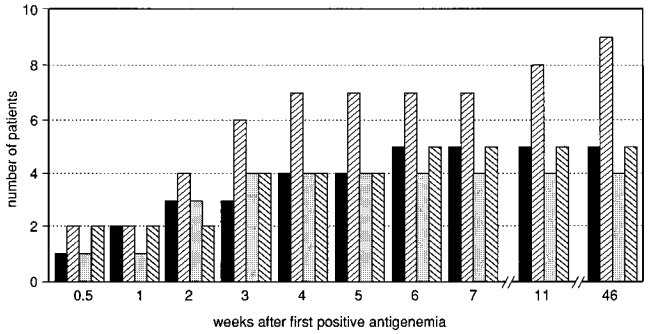

FIG. 2. Appearance of a significant rise in IgG antibodies during a secondary CMV infection in weeks after the first positive antigenemia test. The numbers of patients with responses to the four CMV recombinant proteins pp65 (■), p52 (▨), IE1 (▩), and IE2 (▧) were determined. The results for one patient without a positive antigenemia test were omitted from this figure.

To compare the antibody response to a specific protein with the antibody response to another protein, the antibody response factor was calculated (see Materials and Methods). It was found that the magnitude and rapidity of the antibody responses against recombinant p52 and pp65 were higher than those against both immediate early proteins. The highest antibody factor to pp65 ranged from <1 to 80 (median, 8); for p52, it ranged from <1 to 250 (median, 20); for IE1, it ranged from <1 to 10 (median, <1); and for IE2, it ranged from <1 to 20 (median, 3). When the results of the multispecific ELISA were compared with those of the recombinant protein-based ELISAs, it was noticed that high antibody levels in the former ELISA correlated with high levels to p52 and pp65.

Antigen capture ELISA. The same sera tested in the recombinant protein ELISAs were also tested in the antigen capture ELISAs specific for p52, pp65, and IE2. In the latter, antibodies directed to epitopes exposed on naturally occurring, i.e., native, antigens were detected preferentially. Antibodies directed to the IE1 protein could not be measured in this assay since a monoclonal antibody specific for this protein was not available.

In the sera of patients with primary CMV infections, IgG antibodies to all three CMV proteins could be detected. All five patients developed antibodies to p52, while four of five developed antibodies to pp65 as well as to IE2. Antibodies detected in the antigen capture ELISA appeared simultaneously with the antibodies found in the recombinant protein-based ELISA. The sera of patients with secondary CMV infections were also tested in the antigen-catching ELISAs for conformational epitopes of pp65, p52, and IE2. Significant antibody rises in both ELISA systems were found to occur at the same time. However, some sera showing no reactivity against a certain protein in the recombinant protein-based ELISA were found to be positive in the antigen capture ELISA. An example of such a patient serum is shown in Fig. 4. All individuals eventually had significant antibody increases to p52, and 9 of 10 had antibody rises to pp65 and IE2 as assessed in the capture ELISA.

When the antibody response factors were calculated for this ELISA, the highest responses were found to p52: the calculated numbers for this response ranged from 2 to 250, with a median of 50. For the pp65 antigen capture ELISA, the antibody factors ranged from 4 to 66, with a median of 15, and for the IE2 antigen capture ELISA, a range of 6 to 80 was seen,

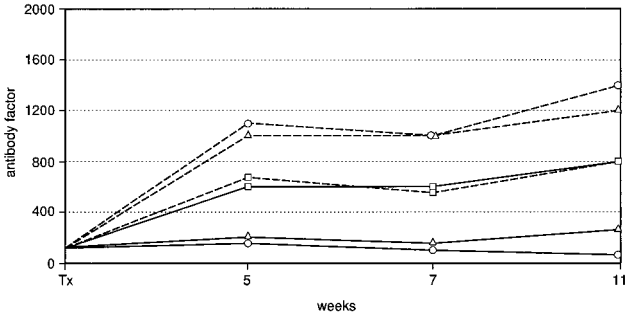

FIG. 4. An example of results of a representative patient with low antibody responses to the recombinant proteins pp65 (—□—), p52 (—△—), and IE2 (—○—) and higher responses to the naturally occurring proteins pp65 (···□···), p52 (···△···), and IE2 (···○···). Tx, day of transplantation.

with a median of 27. Thus, the antibody factors found in the three different antigen capture ELISAs were all higher than those found in the recombinant protein-based ELISAs. The IE2 responses especially differed in the two ELISA systems.

Relationship with antigenemia test. The results of the anti-recombinant CMV protein antibody measurements were related to the maximum level of antigenemia during active CMV infection. No relationship was seen in the five patients with primary CMV infections, with neither the IgM nor the IgG levels. However, in patients with secondary infections, an inverse relationship between the IgG levels to the recombinant proteins and the maximum number of CMV-positive cells was seen (Fig. 5). The highest antibody levels were found in patients with low maximum numbers of CMV-positive leukocytes in the CMV antigenemia test, and, vice versa, patients who had no or very low antibody levels to the four recombinant proteins had the highest numbers of CMV-positive cells. This relationship was found to be statistically significant for the anti-p52 antibodies ($P = 0.02$).

In contrast, no correlation between the IgG antibody levels in the antigen capture ELISA and the maximum levels of CMV antigenemia could be found. Only one patient who was found to have the highest number of positive cells in the CMV antigenemia test had no antibody responses to the naturally occurring pp65 and IE2.

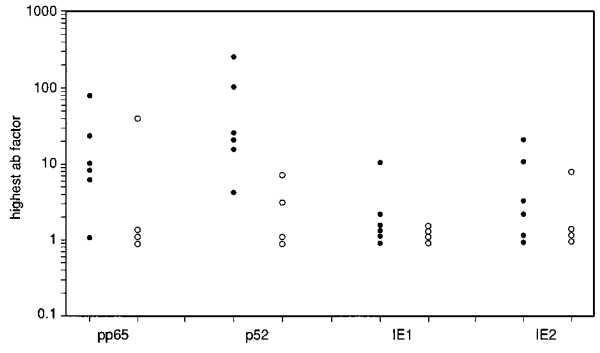

FIG. 5. Relationship between the viral load and the magnitude of the antibody responses to the different recombinant proteins. Symbols: ●, patients with low maximum numbers of CMV-positive cells by the antigenemia test (i.e., 0, <1 [two times], 1, 4, and 6); ○, patients with high maximum numbers of CMV-positive cells by the antigenemia test (i.e., 23, 24, 40, and 156).

DISCUSSION

When antibody responses to four CMV proteins were studied in lung transplant recipients, the following was found. After a primary CMV infection in most patients, IgM antibodies are directed against p52 and pp65. Antibodies against the IE1 and IE2 proteins were detected less often, indicating that these are less immunogenic. Although IgM antibodies to one or more of the recombinant proteins could be found in most patients, few had IgG antibodies to these proteins. During a secondary infection, the antibody responses to the different recombinant CMV proteins showed considerable variation, but higher responses to p52 and pp65 were found in comparison with those against the immediate early proteins. The anti-p52 response was especially high. The higher responses to pp65 and p52 are in agreement with our previous findings (16) and those of others who found intense and rapid reactivity against polypeptides with molecular sizes of 66 and 50 kDa (3, 4) or when a p52 fusion protein was used as antigen (9). However, the lower antibody responses to IE1 do not correspond with the high cellular immune response against this protein (1).

Some patients who developed anti-CMV-specific antibodies as detected in the multispecific ELISA had no or very low antibody levels to the four recombinant proteins. Although antibodies specific for other CMV proteins were also detected in the multispecific ELISA, we found that serum samples of patients that were negative in the recombinant protein-based ELISAs appeared to be positive in the matching antigen-catching ELISAs. This finding suggests that these patients developed antibodies predominantly to epitopes exposed on naturally occurring proteins (conformational epitopes) instead of epitopes present on nonglycosylated or nonphosphorylated denatured recombinant proteins (linear epitopes). In general, antibody responses to conformational epitopes were higher than they were to linear epitopes, but they appeared at the same time.

In all patients with primary CMV infections, significant IgG antibody responses to p52 were found in the antigen capture ELISA, and all but one had antibodies to pp65 and IE2. However, in the recombinant protein ELISA, weak antibody responses to these proteins were found in not more than two patients. This suggests that during a primary CMV infection, a major part of the antibody response is directed to the conformational epitopes. During a secondary CMV infection, high antibody levels were found in nearly every patient in the capture ELISA as well. From the results of the recombinant protein-based ELISAs, it appeared that only anti-p52 antibodies could be found in all patients and that this recombinant protein also induced the highest antibody responses. These results indicate that, in the case of p52, a strong antibody response against both conformational and linear epitopes can be raised, while in the case of pp65 and IE2, antibodies show a stronger response to conformational epitopes. The anti-IE2 response detected in the capture ELISA especially appeared to be higher than the one found in the other ELISA.

To correlate the findings of both ELISA systems with a parameter known to reflect the clinical CMV status of a patient, results were related to the maximum number of positive cells found in the CMV antigenemia test. A positive antigenemia test has been shown to correspond to the period of viremia (13) and to correlate with disease symptoms since the duration of antigenemia was longer and the number of positive cells was higher in symptomatic than in asymptomatic infections (12). With patients with primary CMV infections, no correlation between the results with ELISA and those of the antigenemia test was seen. However, one patient who had a

maximum antigenemia test of 4 positive cells per 50,000 leukocytes, which is very low for a primary CMV infection, had an early antibody response as tested in the recombinant protein-based ELISA, predominantly to pp65 and IE2, both IgM and IgG classes. An interesting finding was that in patients with secondary CMV infections, the level of antibodies to linear epitopes was inversely correlated to the level of antigenemia but the level of antibodies to conformational epitopes was not. Although this was statistically significant only for the anti-p52 response, three patients with the highest antigenemia had no detectable or very low antibody responses to all four recombinant proteins but considerable levels detected by the antigen-catching ELISA. This finding may suggest a biological function for antibodies detecting intracellularly located, linear antigens or parts thereof. The function of such antibodies is difficult to explain, especially since the antigen recognized is located in the cell. It may be that such antibodies are helpful in clearing released CMV proteins or parts thereof from CMV-infected cells that have been lysed by cytotoxic T cells. If this should be the case, then antibodies may play a secondary part in the immunity against CMV infection, i.e., only after the cellular immunity has fulfilled its task.

In conclusion, antibodies directed against the four different CMV-induced proteins generally appeared at the same time after infection. The highest antibody levels were found to p52 and pp65 as compared with those to the immediate early antigens detected in both the recombinant and the capture ELISA systems. When the antibody responses measured by these two assays were compared with each other, it was noticed that conformational epitopes induce higher antibody responses and responses in more patients than linear epitopes. However, antibodies to the latter could be more clinically relevant since, in this group of lung transplant recipients, the occurrence of antibodies to linear epitopes appears to be inversely related to the viral load during a CMV infection. A possible pathophysiological significance of antibodies against linear epitopes requires further study.

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