Humoral Immune Response to Human Cytomegalovirus in Patients Undergoing Percutaneous Transluminal Coronary Angioplasty

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Possible causal relations between prior human cytomegalovirus (HCMV) infection and atherosclerosis and between HCMV reactivation and restenosis after coronary angioplasty have been suggested. We investigated patterns of antibodies directed to HCMV in 112 patients undergoing percutaneous transluminal coronary angioplasty (PTCA) and in a group of sex- and age-matched controls (blood donors without evidence of atherosclerosis). Levels of antibodies to HCMV were measured by enzyme-linked immunosorbent assay (ELISA) of serum samples drawn before and 5 weeks after PTCA. To further differentiate the humoral immune response, we specifically tested antibody reactivity towards four single HCMV proteins (IE2, p52, pp150, and pp65) by recombinant ELISAs. We found that 73% of PTCA patients and 69% of sex- and age-matched controls were seropositive for HCMV (odds ratio, 1.2 [not significant]). The corresponding odds ratios for matched pairs ranged in the recombinant ELISAs from 1.2 to 1.4. Patients had more often high titers of anti-HCMV antibodies (11 versus 4%; odds ratio = 3.3 [0.9 to 15.2]; P = 0.052) and high titers of anti-pp150 antibodies (13 versus 4%; odds ratio = 6.0 [1.3 to 38.8]; P = 0.008). Anti-HCMV immunoglobulin M antibodies were not detected in any patient. There was no evidence of acute HCMV reactivation after PTCA, since the titers of antibodies to the investigated recombinant proteins did not increase at 5 weeks after PTCA. Our results show a limited association between prior HCMV infection and coronary artery disease. We infer that positive anti-HCMV titers are not a major risk factor at the time of disease manifestation. However, this study cannot rule out a possible role of HCMV at earlier stages of the atherosclerotic process. Recombinant ELISAs provide a valuable tool for investigating the antiviral immune response.

There is evidence that ubiquitous viruses such as members of the human herpes virus group may be involved in the pathogenesis of atherosclerosis. This evidence emerges from animal models and from pathologic and seroepidemiologic studies in humans. In animal models, herpesviruses provoke atherosclerotic lesions, alter cholesterol metabolism in smooth muscle cells, and elicit the expression of cytokines and cellular adhesion molecules from the vascular wall (6, 9, 20). Studies on human atherosclerosis revealed an association with human cytomegalovirus (HCMV) but not with other members of the herpesvirus group. Most of these investigations have been pathologic studies of arterial tissues taken from patients undergoing vascular operation or from autopsies. HCMV antigens (15) and HCMV DNA (18) have been detected in smooth muscle cell cultures derived from atherosclerotic plaques. By PCR, a high percentage (90%) of atherosclerotic arterial walls were shown to be latently infected with HCMV (10). There was especially strong clinical and experimental evidence indicating the role of HCMV infection in the development of accelerated aortic atherosclerosis (8, 12). Few data about the seroepidemiology of HCMV infection in atherosclerotic patients have been published. Adam et al. described a higher seroprevalence of antibodies to HCMV in vascular surgery patients than in controls, and this association was strongest in subjects with high antibody titers (1). However, these results were not confirmed by others (3, 5). Two studies have suggested a weak correlation between HCMV seropositivity and carotid artery thickening measured by ultrasound, which is regarded as a measure for early, preclinical atherosclerosis (17, 19). The latter of these studies demonstrated thickened artery walls in individuals who were seropositive for HCMV 10 to 15 years earlier, when blood was obtained for another study and was frozen and saved. Taken together, the published data from epidemiologic studies do not allow a conclusive answer and the association remains tenuous.

More recently, a direct link between HCMV infection and restenosis after coronary angioplasty has been suggested (21). In approximately one-third of restenosis lesions the tumor suppressor gene product p53 accumulated, and essentially the same samples were HCMV DNA positive by PCR. In vitro transfection studies supported a possible inactivation of the p53 function by IE2, one of the viral immediate early gene products. In this way, HCMV may contribute to the development of restenosis by conferring a selective growth advantage on infected smooth muscle cells or by blocking apoptosis in these cells (26). This finding raised the possibility that a similar mechanism might underlie primary atherogenesis.

The present study was initiated to evaluate further the suspected association between atherosclerosis and prior infection...
with HCMV in a group of percutaneous transluminal coronary angioplasty (PTCA) patients and in matched controls. To detect a possible reactivation of latent virus as a result of the angioplasty procedure, levels of antibody to HCMV were also retested 1 month after PTCA. To achieve a better characterization of the humoral immune response, we used a convention-
al multispecific enzyme-linked immunosorbent assay (ELISA) as well as ELISAs based on recombinant viral proteins. These allow the investigation of distinct B-cell responses to single HCMV antigens in comparison to total HCMV reactivity.

MATERIALS AND METHODS

Patient population and blood sampling. A total of 112 consecutive patients (71 men; 41 women; mean age, 62.9 years; range, 34 to 82 years) who were candidates for elective PTCA of a de novo lesion were enrolled for the study. All patients had given written informed consent to participate in this study prior to the PTCA procedure. Blood was drawn immediately before and 5 weeks after PTCA (median, 38 days; range, 17 to 93). Titers of total antibody against HCMV (immunoglobulin G [IgG] and IgM) were determined with fresh serum. Aliquots of serum were stored at −30°C until recombinant ELISAs were performed.

Control group. The control group consisted of randomly selected, sex- and age-matched (maximum difference, 2 years) blood donors, whose samples were obtained from the Department of Blood Transfusion at the University Hospital Groningen, for the age range of 35 to 72 years and of participants in a geriatric study, performed at the University of Leiden, for the age range of 73 to 82 years (13). Since mean ages for the control group was 62 years (range, 35 to 82). Patients and control individuals were all inhabitants of The Netherlands and had similar socioeconomic backgrounds. Exclusion criteria for control individuals were evidence of atherosclerosis (defined as a positive history for myocardial infarction, stroke, angina pectoris, or intermittent claudication) and evidence of autoimmuno-

Cloning and expression of recombinant HCMV antigens in Escherichia coli. The production of the expression constructs containing the pp52 or IE2-cDNA has been described previously (23); pp150 was cloned in the same way. Briefly, DNA fragments coding for the different HCMV antigens were generated by PCR amplification with primers selected from the published sequence of HCMV strain AD169 (2). Plasmid pHM124 (gift of T. Stammering, Erlangen, Germany) containing cDNA of the 84-kDa immediate early antigen or purified DNA of HCMV strain AD169 served as the template for amplification. DNA fragments were cloned into pQE-9 (Qiagen), a vector which enables the inducible expression of polyepitides in fusion with an N-terminally added tag of six histidine residues. Cloned inserts were checked by restriction enzyme analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of induced-cell lysates showed bands of the expected length; the identity of recombinant proteins was confirmed by Western blot analysis with specific monoclonal antibodies. After lysis of induced bacterial pellets in buffer containing 6 mol of guanidine-hydro
dchloride per liter, recombinant proteins were purified by immobilized metal

Cloning and expression of recombinant pp65 in insect cells. The construction of the recombinant baculovirus pp56-HIS-bac will be published elsewhere. Briefly, the coding sequence of pp65 was recloned from prokaryotic expression vector pT7Blue (23) into pFastbac, a shuttle vector of the Bac-to-Bac system (Gibco Life Sciences) to obtain pFastbac. This vector was then transformed into DH-10-bac cells, and after transposon-mediated recombination, bacmid DNA was isolated and transfected into Sf-9 cells by CaCl2 coprecipitation. After preparation of a high-titer virus stock, we used SF-21 cells for protein production. After lysis of the cell pellet under nondenaturing conditions, purification of recombinant pp65 was essentially the same as for prokaryotically expressed proteins.

HCMV serology. (i) Multispecific ELISA. Quantitative determination of total HCMV-specific antibody (IgG or IgM) was performed as described previously (22). Briefly, polystyrene microliter plates (96 well; Greiner) were coated with protein extracts made from late-stage HCMV-infected fibroblasts and with extracts from mock-infected fibroblasts as a control. Serum samples were added in serial twofold dilutions in incubation buffer (0.01 mol of Tris-HCl and 0.3 mol of NaCl per liter, 1% bovine serum albumin, 0.05% Tween 20 [pH 8.0]) and incubated for 45 min at 37°C. Bound immunoglobulins were detected by incubation with IgG-specific peroxidase-labeled conjugate (de Beer, Medicals, Diessen, The Netherlands) for 45 min at 37°C. To block unspecific binding, 5% normal goat serum was added to the conjugate solution. Color was developed with 1,2-

(ii) ELISAs using recombinant proteins. Levels of antibody directed against single HCMV antigens were determined with a recombinant-antigen ELISA as described previously but with some modifications (23). Briefly, polystyrene mic
liter plates (96 well; Greiner) were coated at 4°C for at least 48 h with 100 ng of purified recombinant antigen diluted in 100 μl of 0.1 mol/liter carbonate buffer, pH 9.5, per well. Plates were incubated for 45 min at 37°C with human serum added in serial twofold dilutions. Predilution was 1:100 for IE2 and 1:200 for pp52, pp150, and pp65. Bound immunoglobulins were detected by incubation with IgG-specific peroxidase-labeled conjugate (de Beer, Medicals, Diessen, The Netherlands) for 45 min at 37°C. To block unspecif
cative binding, 5% normal goat serum was added to the conjugate solution. Color was developed with 1,2-

TABLE 1. Characteristics of the patient population (n = 112)*

| Characteristic | Value |
|---------------|-------|
| Sex (M/F)     | 71/41 |
| Age (yr)      | Mean (SD) | 62.9 (10.0) |
|               | Range    | 34–82    |
| Previous ischemic events | |
| Previous CABG | 9     |
| Previous PTCA (other lesion) | 10   |
| Previous myocardial infarction | 42   |
| Lesions (total no.) | 123  |
| LAD           | 39     |
| Cs            | 42     |
| RCA           | 41     |
| LM            | 1      |

* M, male; F, female; CABG, coronary artery bypass graft; LAD, left anterior descending coronary artery; Cs, circumflex artery; RCA, right coronary artery; LM, left main coronary artery.

Reproducibility of the multispecific ELISA was assessed by using pools of known seropositive and seronegative samples as controls on each plate. Test runs were considered acceptable when the values for controls on the actual plate were within 2 standard deviations from the mean of all control values obtained during establishment of this assay. Interassay variability of the controls was 6%.

Lesions (total no.) | 123 |
| LAD | 39 |
| Cs  | 42 |
| RCA | 41 |
| LM  | 1  |

Statistical methods. Statistical analysis was performed with a Systat software package (Systat Inc., Evanston, Ill.). The comparison of cases and controls was estimated by using the pair-matched odds ratio and statistically tested by using McNemar’s test for matched case-control pairs. All tests were two sided. Anti-body titers are reported as medians and compared by the Wilcoxon test for paired samples.

RESULTS

Prevalence of HCMV antibodies in patients and controls.

The distributions of age, sex, and several known risk factors for atherosclerosis in the 112 patients included in the study are shown in Table 1. Levels of antibodies to HCMV in 112 pa
tients undergoing PTCA and in matched controls were mea
sured with a quantitative multispecific ELISA. None of the patients admitted for PTCA had elevated titers of IgM antibody to HCMV as a sign of an active or recent infection. The ELISA showed that 73% of patients and 69% of matched controls were seropositive for HCMV IgG antibodies. The prevalence of antibodies in matched pairs of PTCA cases and controls is shown in Table 2. The odds ratios were calculated...
IMMUNE RESPONSE TO HCMV IN PTCA PATIENTS

In a previous study, the prevalence of high levels of antibodies to HCMV was the most prominent difference between atherosclerotic patients and controls (1). In our study, too, slightly more patients than control subjects had high antibody titers in the multispecific ELISA as well as in all four recombinant ELISAs. This pattern was most distinct in the multispecific ELISA and in recombinant ELISAs against pp150 and pp65. However, differences in median values between cases and controls were minimal. Odds ratios were calculated for frequency of high levels of antibodies to HCMV, which was defined as an antibody titer above 50%. Results are shown in Table 3. The frequency of high pp150 antibody levels in the PTCA patients was significantly higher than that in the controls (13 versus 4%; odds ratio = 6.0 [1.3 to 38.8]; P = 0.008). The difference for the multispecific ELISA was not quite significant (11 versus 4%; odds ratio = 3.3 [0.9 to 15.2]; P = 0.052).

Of our PTCA patients, 34% had experienced a myocardial infarction previous to PTCA. Seroprevalence and antibody titers in this subgroup did not differ significantly from those in the other PTCA patients.

**Evidence against the presence of acute infection and systemic reactivation after PTCA.** From 84 patients we were able to obtain serum samples to monitor HCMV antibody titers 5 weeks after PTCA. During the course of the study, seroconversion of a previously negative patient did not occur and no previously positive patient became negative. Anti-HCMV IgM antibodies, which are usually present only early after acute infection, were not detected in any of the patients at baseline or at the follow-up examination. In seropositive patients, antibody titers did not change significantly as a result of the PTCA procedure. Titers of antibody to the recombinant antigens pp65 and p52 and to HCMV are shown in Fig. 1. Only 15 patients (13%) showed a moderate rise in antibody titers; none of these met the criteria for acute reactivation (titer increase > 100% of the original value). Small increases and variabilities in HCMV antibody titers were often found in samples with cross-reacting substances such as rheumatoid factor. Of patients who showed an increase in HCMV titers, 35% had rheumatoid factor, compared to 13% of all patients.

**Discussion**

The present study was designed as a prospective serological investigation to test the hypothesis that HCMV is involved in the pathogenesis of atherosclerosis. A total of 112 consecutive PTCA patients were compared with a group of sex- and age-matched blood donors. HCMV seroprevalences were 74% in the patient group and 69% in the control group, prevalences similar to those reported in several epidemiologic studies involving subjects of similar age (4). The odds ratio for previous HCMV infection was 1.2 and did not reach statistical significance. Analyzing the immune reaction more specifically by ELISAs using single recombinant viral proteins as target antigens, we found a tendency towards a stronger association. High titers of anti-HCMV antibodies showed a significant association with atherosclerosis. However, in a previous study on cardiovascular surgery patients versus controls, a much stronger association was described (1). In that particular study population, the odds ratio for prior HCMV infection was 3 and a significantly greater percentage of surgical cases than controls had high titers of HCMV antibodies. In this regard, we cannot confirm the strong association between symptomatic atherosclerosis and prior HCMV infection. We found only a tendency towards higher seroprevalence in atherosclerotic patients and only a weak association with high antibody levels.

One could argue that the ELISA used in the present study was not sensitive enough in that it may have failed to identify patients with low titers. However, this does not seem very likely, as we have previously shown that this multispecific HCMV ELISA is very sensitive and reliable and we have used it successfully in a large number of clinical studies (22). Moreover, the most pronounced differences between cases and controls in the cited surgical study were in the subgroup with high HCMV titers.

It seems more likely that PTCA patients differ from the surgical patients of the previously mentioned study with respect to the extent of the atherosclerotic lesions. Patients se-

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**Table 2**

| Controls | Multi-specific | IE2 | p52 | pp150 | pp65 |
|----------|----------------|-----|-----|-------|------|
| Ab<sup>a</sup> | Ab<sup>b</sup> | Ab<sup>a</sup> | Ab<sup>b</sup> | Ab<sup>a</sup> | Ab<sup>b</sup> | Ab<sup>a</sup> | Ab<sup>b</sup> | Ab<sup>a</sup> | Ab<sup>b</sup> |
| Ab<sup>a</sup> | 6 | 29 | 79 | 15 | 27 | 35 | 33 | 32 | 12 | 27 |
| Ab<sup>b</sup> | 24 | 53 | 11 | 7 | 25 | 25 | 23 | 24 | 23 | 50 |
| Odds ratio | 1.2 | 1.4 | 1.4 | 1.4 | 1.2 | 0.492 | 0.432 | 0.196 | 0.226 | 0.572 |
| P value<sup>b</sup> | 0.0075 | 0.655 | 0.336 | 0.0075 | 0.394 |

<sup>a</sup> Ab<sup>a</sup>, antibody negative; Ab<sup>b</sup>, antibody positive. <sup>b</sup> P values were calculated with McNemar's test for matched case-control pairs (two tailed).

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**Table 3**

| Controls | Multi-specific | IE2 | p52 | pp150 | pp65 |
|----------|----------------|-----|-----|-------|------|
| Ab<sup>a</sup> | Ab<sup>b</sup> | Ab<sup>a</sup> | Ab<sup>b</sup> | Ab<sup>a</sup> | Ab<sup>b</sup> |
| Ab<sup>a</sup> | 97 | 10 | 107 | 3 | 81 | 16 | 95 | 12 | 87 | 13 |
| Ab<sup>b</sup> | 3 | 2 | 2 | 0 | 11 | 4 | 2 | 3 | 9 | 3 |
| Odds ratio | 3.3 | 1.5 | 1.5 | 6.0 | 1.4 |
| P value<sup>b</sup> | 0.0052 | 0.655 | 0.336 | 0.0075 | 0.394 |

<sup>a</sup> Ab<sup>a</sup>, antibody (high titers) negative; Ab<sup>b</sup>, antibody (high titers) positive. <sup>b</sup> P values were calculated with McNemar's test for matched case-control pairs (two tailed).
lected for PTCA have a localized stenosis in one or more coronary arteries, which may be a different situation from more disseminated forms of atherosclerosis in vascular surgery patients. If it is true that the presence of HCMV promotes the development of vessel wall injury and atherosclerosis, a stimulation of antibody production to higher levels may be expected in patients with more extended forms of atherosclerosis.

There are several limitations with serological studies. A positive antibody test is only indicative of prior infection and provides limited information on time of infection, or repeated infection. Additionally, after primary infection, titers of antibody to HCMV may fall and may become undetectable despite persistence of the virus. In a recent study, HCMV DNA detection in vessel walls was compared to HCMV serology, but there was no correlation between presence of the virus in the tissue and presence of antibodies in serum (16). Moreover, HCMV in the wall of blood vessels may only be a fraction of the total HCMV body load. These issues may be relevant in assessing the relationship between HCMV and atherosclerotic disease.

A crucial problem in clinical studies dealing with atherosclerosis is the selection of a proper control group. From autopsy studies, it is known that essentially every adult in Western countries has more or less extended atherosclerotic lesions in the vessel walls. Therefore, differences between cases and controls are more gradual than absolute. In the present study, the healthy blood donors recruited as controls had no history or clinical evidence of atherosclerosis, which was confirmed by a standardized interview and a physical examination. We did not find a significantly higher HCMV seroprevalence in PTCA patients than in these controls.

In addition to the sensitive multispecific ELISA, we developed recombinant ELISAs to investigate distinct B-cell responses to selected single viral proteins. IE2, one of the virus' immediate early proteins, is known to be a promiscuous transactivator of many viral and cellular genes. This antigen is proposed to promote restenosis by the functional inactivation of the tumor suppressor gene product p53 (21). As is known from previous studies, levels of antibody against IE2 in healthy adults are infrequent and low (23). In our patient population, 21% of the subjects showed specific antibody reactivity against this protein. Most important, levels of antibody to IE2 did not increase during the ensuing 5 weeks. The virus seems to have developed efficient mechanisms to protect these important regulatory proteins from recognition by the host immune system (7, 25). In any case, immediate early gene expression is an indispensable step in the life cycle of the virus, and if PTCA causes a reactivation of HCMV, the expression of this antigen does not lead to a measurable immune response.

Two other viral proteins, the basic phosphoprotein of 150 kDa encoded by UL32 and the nonstructural DNA-binding protein of 52 kDa encoded by UL44, have repeatedly been shown to elicit a strong and early immune reaction during natural infection (11, 24). In the recombinant ELISAs using these two proteins as target antigens, a more pronounced association was found with coronary artery disease than in the multispecific ELISA. It reached significance at high titers of anti-pp150 antibodies. It is interesting that these recombinant ELISAs in which we use prokaryotically expressed proteins are tests whose results indicate a lower overall seroprevalence than the multispecific ELISA but have a stronger correlation to the clinical status of the patient. Prokaryotically expressed proteins are not posttranslationally modified, and due to the followed procedure they are isolated in a completely denatured (i.e., linearized) conformation. Thus, they are recognized only by antibodies directed to linear epitopes. These antibodies develop late in the course of an immune reaction, often after immunity maturation. High-titer reactivity to prokaryotically expressed proteins, as measured in our recombinant ELISAs, may therefore be a marker for a more chronic infection process.

The lower matrix phosphoprotein pp65 has been recognized as the dominant target of the cytotoxic T-cell response in HCMV infection (14). We have shown previously that pp65 also represents an important target for the humoral immune response (23). However, most of the pp65 immunoreactivity is directed to conformation-dependent epitopes. The pp65 ELISA reached 93% of the sensitivity of the multispecific ELISA, with a correlation coefficient of 0.78 between the two ELISAs. This means that a considerable part of antibody activity measured in the multispecific ELISA is directed against pp65. In PTCA patients, anti-pp65 reactivity was not related to atherosclerosis.
In conclusion, we investigated patterns of antibodies directed to HCMV in PTCA patients before and 5 weeks after the intervention. We found a limited association between prior HCMV infection and coronary artery disease but no measurable systemic reactivation after PTCA. These results agree with the results of most of the foregoing studies (4). Moreover, we used much more refined techniques for the investigation of the humoral immune response. The ELISAs using recombinant p53 and pp150 provide a tool to investigate the antiviral immune response with higher sensitivity and with a better correlation to the clinical status of the patients. The two corresponding genes may be of use for detecting the assumed chronic reactivation in the atherogenic process. In contrast, IE2 was not of value for serological purposes. The pp65 ELISA did not yield additional information, but our results show that eukaryotically expressed pp65 is a first-class candidate to replace the virus and infected cells as an antigenic substrate in the serological evaluation of anti-HCMV antibody.

Taken together, our data do not support an important role for HCMV in the pathogenesis of atherosclerosis, and the question of whether the very weak association between HCMV infection and coronary artery disease may be a consequence rather than a cause of the latter is raised.

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REFERENCES

1. Adam, J. L., Probstfield, J. Burek, C. H. McCollum, J. L. Melnick, B. L. Petrie, K. R. Bailey, and M. E. DeBakey. 1987. High levels of cytomegalo-virus antibody in patients requiring vascular surgery for atherosclerosis. Lancet 1:291–293.

2. Chee, M. S., A. T. Bankier, S. Beck, R. Bohni, C. M. Brown, R. Cerny, T. Hornell, C. A. Hutchison III, T. Kourideres, J. A. Martignetti, E. Preddie, S. C. Satchwell, P. Tomlinson, K. M. Weston, and D. G. Barrell. 1990. Analysis of the protein-coding content of the sequence of human cytomegalovirus strain AD169. Curr. Top. Microbiol. Immunol. 154:125–169.

3. Cour, M. I., F. J. Lopez-de Atalaya, L. Palau, E. Fernandez Contreras, and C. Perezagua. 1989. Lack of serological association between herpesvirus and atherosclerosis. Lancet 1:679–682.

4. Danesh, J., R. Collins, and R. Peto. 1997. Chronic infections and coronary heart disease: is there a link? Lancet 350:430–436.

5. Dummer, S. A. Lee, M. K. Breining, R. Karmos, M. Ho, and B. Griffith. 1994. Investigation of cytomegalovirus infection as a risk factor for coronary ath- erosclerosis in the explanted hearts of patients undergoing heart transplantation. J. Med. Virol. 44:305–309.

6. Fabricant, C. G., J. Fabricant, M. L. Lichten, and C. R. Minick. 1978. Virus-induced atherosclerosis. J. Exp. Med. 148:335–340.

7. Gilbert, M. J., S. R. Riddell, C. R. Li, and P. D. Greenberg. 1993. Selective interference with class I major histocompatibility complex presentation of the major immediate-early protein following infection with human cytomegalovirus. J. Virol. 67:3461–3469.

8. Grittian, M. T., C. E. Moreno-Cabral, V. A. Starnes, P. E. Oyer, B. E. Stinson, and N. E. Shumway. 1989. Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. JAMA 261:3561–3566.

9. Hau, D. P., C. G. Fabricant, M. L. Lichten, and J. Fabricant. 1986. Virus-induced atherosclerosis herpesvirus infection alters acetic cholesterol metabolization and accumulation. Am. J. Pathol. 122:62–70.

10. Hendrix, M. G., M. M. Saltimans, C. P. van Boven, and C. A. Bruggeman. 1995. High prevalence of latent cytomegalovirus infection in patients suffering from grade III atherosclerosis. Am. J. Pathol. 146:23–28.

11. Landini, M. P., T. Lazzaro, G. T. MAine, A. Ripaldi, and R. Flanders. 1995. Recombinant monoc- and polyaotigens to detect cytomegalovirus-specific immunoglobulin M in human sera by enzyme immunoassay. J. Clin. Microbiol. 33:2535–2542.

12. Lemström, K., P. Koskinen, L. Krogerus, M. Daemen, C. Bruggeman, and P. Häyry. 1995. Cytomegalovirus antigen expression, endothelial cell proliferation, and intimal thickening in rat cardiac allografts after cytomegalovirus infection. Circulation 92:2594–2604.

13. Lifghart, G. J., X. Corberand, H. G. Geertzen, A. E. Meinders, D. L. Knook, and W. Hijnmans. 1990. Necessity of the assessment of health status in human immunonongenetics studies: evaluation of the SEINEUR prospective study. Mech. Ageing Dev. 55:90–105.

14. McLaughlin-Taylor, E., H. Pande, S. J. Forman, B. Tanamachi, C. Li, J. A. Zaia, D. Greenberg, and S. R. Riddell. 1994. Identification of the major late human cytomegalovirus matrix protein pp65 as a target antigen for CD8+ virus-specific cytotoxic T lymphocytes. J. Med. Virol. 43:103–110.

15. Melnick, J. L., G. R. Dressman, C. H. McCollum, B. L. Petrie, J. Burek, and M. E. DeBakey. 1983. Cytomegalovirus antigen within human arterial smooth muscle cells. Lancet 1:644–647.

16. Melnick, J. L., C. H. Hu, J. Burek, E. Adam, and M. E. DeBakey. 1994. Cytomegalovirus DNA in arterial walls of patients with atherosclerosis. J. Med. Virol. 42:170–174.

17. Nieto, F. J., E. Adam, P. Sorlie, H. Farzailedan, J. L. Melnick, G. W. Cowen, and M. Szeklo. 1996. Cohort study of cytomegalovirus infection as a risk factor for carotid intimal-medial thickening, a measure of subclinical atherosclerosis. Circulation 94:922–927.

18. Petrie, B. L., J. L. Melnick, E. Adam, J. Burek, C. H. McCollum, and M. E. DeBakey. 1987. Nucleic acid sequences of cytomegalovirus in cells from human arterial tissue. J. Infect. Dis. 155:158–159.

19. Sorlie, P. D., E. Adam, S. L. Melnick, A. Folsom, T. Skelton, L. E. Chambers, R. Barnes, and J. L. Melnick. 1994. Cytomegalovirus/herpesvirus and atherosclerosis: the ARIC Study. J. Med. Virol. 42:33–37.

20. Span, A. H., G. Grauls, F. Bosman, C. P. van Boven, and C. A. Bruggeman. 1992. Cytomegalovirus infection induces vascular injury in the rat. Atherosclerosis 93:41–52.

21. Speir, E., R. Modali, E.-S. Huang, M. B. Leon, F. Shawl, T. Finkel, and S. E. Epstein. 1994. Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. Science 265:391–394.

22. Van der Giessen, M., A. P. Van den Berg, W. van der Bij, S. Postma, W. J. Van Son, and T. H. The. 1990. Quantitative measurement of cytomegalovi-rus-specific IgG and IgM antibodies in relation to cytomegalovirus anti-genaemia and disease activity in kidney recipients with an active cytomegalovirus infection. Clin. Immunol. 40:56–61.

23. Van Zanten, J., M. C. Harmsen, M. Van der Giessen, W. van der Bij, J. Prop, L. De Leij, and T. H. The. 1995. Humoral immune response against human cytomegalovirus (HCMV)-specific proteins after HCMV infection in lung transplantation as detected with recombinant and naturally occurring pro- teins. Clin. Diag. Lab. Immunol. 2:214–218.

24. Vornagar, R., B. Plachter, W. Hinderer, T. H. The, J. Van Zanten, L. Mater, C. A. Schmidt, H.-H. Sonneborn, and G. Jahn. 1994. Early serodiagnosis of acute human cytomegalovirus infection by enzyme-linked immuno- sorbent assay using recombinant antigens. J. Clin. Microbiol. 32:981–986.

25. Wiertz, E. J., R. Jones, L. Sun, M. Boghy, H. J. Geuze, and H. L. Ploegh. 1996. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. Cell 84:769–779.

26. Zhou, H., Y. Q. Shen, and T. Shenk. 1995. Human cytomegalovirus IE1 and IE2 proteins block apoptosis. J. Virol. 69:7960–7970.