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Original Article

Human cytomegalovirus (HCMV)-specific immunoglobulin E as a serologic marker for HCMV infection in immunocompromised patients

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Summary. An antibody capture assay using an enzyme-linked human cytomegalovirus (HCMV) antigen for the detection of specific immunoglobulin E (IgE) was established. IgG, M, and E responses to HCMV were studied in 497 sera obtained from 44 renal transplant recipients and 51 acquired immunodeficiency syndrome (AIDS) patients. The results were compared with those obtained from 58 HCMV-seropositive healthy individuals. HCMV-specific IgE was detected in 11 (91.7%) renal transplant recipients with primary HCMV infection. In contrast, antibodies of the IgG and IgM classes were detected in only 6 (50.0%) of these patients. Specific IgE was detected in 10 (90.9%) out of 11 renal allograft recipients suffering from secondary HCMV infection. Significant IgG titer rises and IgM were detected in 2 (18.2%) and 6 (54.6%) of these patients, respectively. IgG titer rises and IgM and IgE antibodies were seen in 5 (12.2%), 1 (2.4%) and 18 (43.9%) AIDS patients respectively. All healthy immunocompetent HCMV-seropositive individuals were tested IgE negative. The results obtained in our study indicate that IgE against HCMV is a more reliable serologic marker for primary and secondary HCMV infection than IgM in immunocompromised individuals, especially in organ transplant recipients, since it is not affected by the prophylactic application of HCMV hyperimmune globulin preparations.

Key words: Primary human cytomegalovirus infection – IgE antibody capture enzyme-linked immunosorbent assay – Renal transplant recipients – Acquired immunodeficiency syndrome patients

Human cytomegalovirus (HCMV) is a major cause of life-threatening disease in immunocompromised patients. HCMV antibody-negative renal transplant recipients (RTR) receiving grafts from seropositive donors are at a high risk of acquiring severe HCMV infection [4, 6, 16]. In kidney transplant patients, overt cytomegalic disease is associated with prolonged fever, diffuse pulmonary infiltrates, retinitis, abnormal liver function, hematologic abnormalities as leukopenia and thrombocytopenia, and inversion of the CD4/CD8 T lymphocytes ratio. Consequently, 20% of renal graft rejections and 25% of all mortality in these patients are attributable to HCMV infection [8].

Earlier studies [12, 15] demonstrated that a serologic response to HCMV infection can usually be detected before the virus can be isolated. However, the detection of HCMV immunoglobulin (Ig)G antibodies in seronegative RTR is no longer a reliable marker for HCMV seroconversion since the prophylactic application of HCMV hyperimmune globulin is used for the prophylactic teatment of HCMV infection in seronegative organ transplant recipients. Unfortunately, immunocompromised patients often fail to produce IgM antibodies to HCMV, even during symptomatic infections. Numerous attempts have been made to improve the serologic diagnosis in these patients by the determination of specific immunoglobulins other than IgG or IgM [14].

In order to investigate the diagnostic benefit of specific IgE antibodies as a serologic marker for an active HCMV infection, an antibody-capture enzyme-linked immunosorbent assay (ELISA) using commercially available monoclonal anti-IgE coated tubes was developed. The assay was evaluated by the results obtained from 497 sera drawn from renal allograft recipients, from acquired immunodeficiency syndrome (AIDS) patients with HCMV reactivation, and from healthy controls.

Table 1. Clinical outcome of HCMV disease in RTR and AIDS patients

Patient group	n	HCMV (infection/ disease)	Clinical manifestations of HCMV disease						
			Fever of indeterminate localisation	Leukopenia	Transplant rejection	Interstitial pneumonia	Retinitis	Hepatitis	Colitis
HCMV-seronegative RTR	25	12/ 7	3	4					
HCMV-seropositive RTR	19	10/ 8	2	2	1	1		2	
AIDS patients	51	41/26	11			10	4		1
Total	95	63/41	16	6	1	11	4	2	1

AIDS = acquired immunodeficiency syndrome; HCMV = human cytomegalovirus; RTR = renal transplant recipients

Methods

Antibody-capture ELISA for detection of HCMV IgE antibodies

Lyophilized peroxidase-labeled HCMV antigen (Medac, Hamburg, FRG) was diluted according to the manufacturer's recommendations in 2.5 ml phosphate buffered saline (PBS) with 0.05% Tween 20 and 0.001% thimerosal (PBS-TT). Tubes with covalently bound, monoclonal mouse anti-human IgE antibody (Pharmacia Diagnostics, Sweden) were washed with 1 ml washing solution. Then 50 µl of patient serum was added. After incubation overnight at 4° C, the tubes were washed three times with 1 ml PBS-TT. A 50-µl aliquot of enzyme-labeled HCMV was added to each tube. After 60 min incubation at room temperature, the tubes were again washed 3 times with 1 ml PBS-TT. Then 50 µl of substrate solution (1 tablet of ortho-phenylenediamine diluted in 5 ml solvent; Behringwerke, FRG) were added to each tube. After 10 min of incubation in the dark at room temperature, the reaction was stopped by adding 0.1 ml of 2 M H_2SO_4 to each tube. Absorbance was read by photometry (ELISA Processor, Behringwerke) at 492 nm with 650 nm as the reference wavelength. The cut-off level was determined as the mean absorbance plus 3 standard deviations for 58 sera from healthy HCMV-seropositive individuals without clinical signs of HCMV infection and without viruria. The cut-off level was an absorbance of 0.282. The interassay variation of the IgE-capture ELISA was determined on 10 consecutive days with paired sera from 2 RTR seronegative for HCMV, 2 RTR with primary HCMV infection, and in each serum of 2 healthy individuals known to be seropositive of HCMV without recent infection.

Indirect ELISA for the detection of HCMV IgG and IgM antibodies

HCMV IgG and IgM antibody detection was performed with the commercially available ELISA kit Enzygnost-Zytomegalie (Behringwerke). All sera were processed as previously described [2, 3]. Serological proof of an HCMV infection was defined as a four-fold HCMV-specific IgG titer rise which was not related to a passive immunization. An IgM titre superior to 1/40 was considered as indicative of an active HCMV infection.

HCMV immediate early antigen detection in human foreskin fibroblasts (shell vial culture)

Shell vial culture, including HCMV immediate early antigen detection in human foreskin fibroblasts, was performed as described elsewhere [18].

Patient population

A total of 497 sera were available from 44 RTR, 51 persons suffering from AIDS, and 58 healthy individuals. Twenty-five RTR were seronegative for HCMV before renal transplantation. Twenty-one of the HCMV-seronegative patients received renal allografts from donors seropositive for HCMV; 17 of them were given prophylactic HCMV hyperimmune globulin post-transplantation. A total of 19 RTR were HCMV-seropositive before transplantation. All patients were placed on a maintenance immunosuppressive regimen consisting of prednisolone and cyclosporine. A total of 6–8 consecutive sera were studied from each RTR at a mean ($\pm$ SD) interval of 15.2 ± 8.0 days posttransplantation. Twelve HCMV-seronegative RTR and 11 initially seropositive RTR had evidence of primary HCMV infection and recurrence, respectively. HCMV disease was diagnosed in 7

Table 2. Comparison of serologic testing for immunoglobulins (Ig) G, M, and E in renal transplant recipients (RTR) and AIDS patients

Patient group	n	Patients with HCMV infection disease	Serology		
			IgG[a]	IgM[a]	IgE[b]
HCMV-seronegative RTR	25	12 (7)	6 (4)	6 (6)	11 (7)
HCMV-seropositive RTR	19	11 (8)	2 (2)	6 (6)	10 (8)
AIDS patients	51	41 (26)	5 (5)	1 (1)	18 (18)
Total	95	64 (41)	13 (11)	13 (13)	39 (33)

[a] Seroconversion or fourfold titer raise
[b] Optical density >cut off

initially seronegative RTR and in 8 seropositive RTR (Table 1).

Also enrolled in the study were 51 AIDS patients (stage IV C_1 of HIV infection according to CDC criteria). Of these patients, 41 were suffering from HCMV infection. A total of 2–3 consecutive sera were studied from each AIDS patient at a mean ($\pm$SD) interval of 17.3 ± 11.8 days. Twenty-six AIDS patients presented clinical signs of HCMV disease (Table 1).

Shell vial culture was performed in renal allograft recipients and AIDS patients when HCMV infection or disease were suspected.

Fifty-eight sera from 58 healthy individuals known to be seropositive for HCMV without recurrent active HCMV infection were included in the study as a control group. All sera were stored at $-20°$ C until used.

Criteria for HCMV infection and disease

Primary HCMV infection was diagnosed when a HCMV-specific IgG seroconversion (not related to a passive immunization) was detected, an IgM response against HCMV was documented, or HCMV was isolated from the urine, induced sputum, or bronchoalveolar lavage fluid (BAL).

Secondary (recurrent) HCMV infection was defined as a fourfold IgG titer rise and/or detection of specific IgM and/or virus isolation from a HCMV-seropositive patient.

HCMV disease was defined as HCMV infection accompanied by one or more of the following criteria: (i) fever of $>38.5°$ C for at least 3 days, (ii) abnormal liver function (serum glutamic-oxaloacetic or glutamate pyruvate transferase, SGOT or SGPT>40 IU/ml), (iii) leukopenia (leukocytes $<3500/mm^3$), (iv) thrombocytopenia (thrombocytes $<100000/mm^3$), (v) HCMV retinitis, (vi) cultural and clinical evidence of organ involvement

(HCMV interstitial pneumonia, HCMV hepatitis, and HCMV colitis).

Results

To reduce the influence of test variation on the results of the IgE-antibody capture ELISA, only one batch each of HCMV conjugate, control conjugate, and unlabeled control antigen was used throughout the entire study. Consequently, the interassay variation was very low (<10%).

A dose-response relationship was performed by serial dilution of 2 HCMV-specific IgE-positive and 2 HCMV-specific IgE-negative sera. Although HCMV-specific IgE antibodies could be detected in one positive serum sample diluted 1:320, the assay was routinely performed with undiluted serum samples, according to the manufacturer's recommendations, in order to increase the sensitivity of the assay.

The interfering effect of HCMV hyperimmune globulin, HCMV-specific IgM and HCMV-specific IgA antibody on the IgE-capture ELISA was also investigated. HCMV hyperimmune globulin, HCMV-specific IgM- and HCMV-specific IgA-positive sera gave the same absorbance values for HCMV-specific IgE as the sera of the healthy control group.

As shown in Tables 1 and 2, 67.4% of the 95 immunocompromised patients investigated were suffering from HCMV infection. HCMV disease was present in 41 (43.2%) patients. The clinical manifestations attributable to HCMV are depicted in detail in Table 1.

The highest percentage of reactivity to HCMV-specific antigens was observed with IgE (60.9 ± 13.0, $P=95\%$). In contrast, a significant IgG titer and antibodies of the IgM class were detected in 20.3 ± 9.3 ($P=95\%$) patients. Serologic testing for IgE against HCMV detected primary infection in

Table 3. Comparison of IgG, M, and E for the detection of primary cytomegalovirus infection (disease)

Patient group	n	Cytoglobin	Patients with HCMV infection	Serology IgG[a]	IgM[a]	IgE[b]
25 seronegative renal transplant recipients						
HCMV-positive renal allograft	17	Yes	7 (4)	0 (0)	3 (3)	7 (4)
HCMV-positive renal allograft	4	No	3 (2)	3 (2)	2 (2)	3 (2)
HCMV-negative renal allograft	4	No	2 (1)	3 (2)	1 (1)	1 (1)
Total	25		12 (7)	6 (4)	6 (6)	11 (7)
19 seropositive renal transplant recipients						
HCMV-positive renal allograft	3	Yes	2 (1)	1 (1)	2 (2)	1 (1)
HCMV-positive renal allograft	16	No	9 (7)	1 (1)	4 (4)	9 (7)
Total	19		11 (8)	2 (2)	6 (6)	10 (8)

Cytoglobin = HCMV hyperimmune globulin
[a] Seroconversion or fourfold titer raise
[b] Optical density > cut off

seronegative RTR (91.7 ± 30, $P = 95\%$) as well as recurrent infection in seropositive RTR (90.9 ± 32.2, $P = 95\%$). Only 18 (43.9 ± 15.4, $P = 95\%$) AIDS patients presented with increased HCMV-specific IgE levels.

HCMV antibody response in renal transplant recipients

The results obtained by serologic testing for IgG, M and E in RTR are depicted in Table 3. Within 26–63 days after transplantation, 12 initially HCMV-negative patients were experiencing primary HCMV infection. IgE, G and M were detected in 11 (91.7%), 6 (50.0%), and 6 (50.0%) of these patients. All the RTR suffering from HCMV infection were presenting with viruria at variable time intervals.

In 7 of the RTR with primary infection, HCMV disease was apparent. Increased HCMV-specific IgE levels were detected in all the 7 patients at a mean time interval of 13.5 days (range 10–24 days) after the appearance of clinical symptoms. Six of these patients who developed HCMV-specific IgE antibodies also showed HCMV-specific IgM after 16.5 days (range 12–30 days) following the appearance of clinical symptoms of primary HCMV infection. HCMV-specific IgE could be detected up to 385 days postinfection in 1 patient. HCMV-specific IgG seroconversion was seen in 4 of these patients not undergoing HCMV hyperimmune globulin prophylaxis.

In 5 out of these 12 seronegative transplant recipients, the primary HCMV infection was not associated with disease. A fourfold rise in HCMV-specific IgG was documented in 2 patients of this group not undergoing HCMV hyperimmune globulin therapy. HCMV-specific IgE antibodies were detected in 4 of these patients. Only 1 renal transplant recipient with asymptomatic HCMV infection showed a specific IgM response.

A total of 13 seronegative transplant recipients showed no clinical and serological signs related to primary HCMV infection. All of them were found to be seronegative for HCMV-specific IgE and IgM.

Increased levels of IgE were seen in 10 out of 11 HCMV-positive RTR suffering from recurrent HCMV infection. In contrast, antibodies of the IgM class and increased IgG levels were detected in only 6 and 2 patients, respectively. In 1 out of the 10 patients with recurrent HCMV infection, a specific IgE antibody was the only serologic marker of HCMV infection to be detected. In this patient, HCMV infection was confirmed by virus isolation from one urine sample in the shell vial culture. IgE, G, and M were detected in 8, 2, and 6 seropositive RTR suffering from HCMV disease, respectively.

HCMV antibody response in AIDS patients

As shown in Table 1, 41 out of 51 AIDS patients were suffering from HCMV infection, confirmed by HCMV detection in urine, BAL, and saliva samples by using the shell vial culture technique. Significant titer rises in HCMV-specific IgE antibodies could be observed in 18 out of 26 HIV-

positive patients with HCMV disease. Only 1 of these patients developed specific HCMV-specific IgM antibodies. A fourfold titer rise of IgG was detected in 5 AIDS patients.

HCMV antibody response in healthy individuals

No increased HCMV-specific IgM and IgE antibody levels were observed.

Discussion

The aim of this study was to develop an HCMV-specific IgE antibody capture ELISA for the detection of HCMV-specific IgE in immunocompromised patients and to evaluate it as a serologic marker for the laboratory diagnosis of HCMV infection.

Antibody capture ELISA using enzyme-labeled HCMV antigen has been found to be a sensitive and valuable technique for the detection of HCMV-specific IgG, IgM, IgA, and IgE [1, 11, 13, 14, 19]. The direct ELISA using enzyme-labeled antigen is not impaired by rheumatoid factor or competitive IgG antibodies [8, 17]. Nonspecific reactions, i.e., reactivity in sera against cellular antigens in the HCMV conjugate, have been excluded by using control conjugate.

Sera obtained from the HCMV-positive healthy control group showed absorbances up to 0.136. In order to optimize the specificity of the assay, the cut-off was determined as 2 times the mean absorbance of the healthy control group (0.282).

HCMV-specific IgE antibodies were detected in 91.7% of the initially seronegative renal allograft recipients with primary HCMV infection. Serologic testing for specific IgE detected recurrent HCMV infection in 90.9% of the seropositive RTR and 43.9% of the AIDS patients. In agreement with Van Loon et al. [19], these data confirm that in primary as well as in secondary infection increased HCMV-specific IgE levels can be observed.

Compared with IgG and IgM against HCMV, the IgE class antibody can be regarded as a superior serologic marker for the diagnosis of HCMV infection, since rises in specific IgG and IgM could be demonstrated in only 20.3% of the patients with HCMV infection. These data confirm previous studies suggesting that organ transplant recipients fail to produce IgM antibodies directed against HCMV [1, 11, 17].

AIDS patients were included in this study because the severe immunosuppression caused by viral infection of the lymphocytes might influence the

serologic response to HCMV [7]. In 69.2% of the AIDS patients with clinical evidence of HCMV recurrence, IgE against HCMV was present. Serologic testing for IgM detected HCMV infection in only one AIDS patient. Compared with the shell vial culture which enabled the laboratory diagnosis of HCMV infection in all patients investigated, HCMV-specific IgE detection proved to be less sensitive. Furthermore, AIDS patients suffering from HCMV disease showed less frequently detectable IgE levels than renal allograft recipients.

The IgE antibody response is T-cell dependent, and a disturbed T-cell regulation resulting from a viral infection may be associated with raised serum IgE levels [20, 21]. The consistent diminution of $CD4^+$ lymphocytes as a main cause of human immunodeficiency virus (HIV)-induced immunosuppression might be responsible for unspecific IgE-antibody activation. Other investigators [9] obtained evidence that the IgE response to herpes simplex virus was particularly prominent under conditions in which the number of suppressor T cells is decreased, such as due to immunosuppressive therapy. It is not clear whether this is also true in HIV infections. As shown by our results, the IgE detected in AIDS patients was specific, since virus was isolated from clinical specimens (urine, induced sputum, BAL) obtained from these patients.

The detection of HCMV-specific IgE, IgG, and IgM is not exclusively associated with the occurrence of HCMV disease (Table 2). The detection of specific serological markers for HCMV disease can only be achieved by immunoblot procedures [5, 10]. Nevertheless, in AIDS patients, the presence of IgE antibody against HCMV seems to be associated with HCMV disease.

Present data confirm that HCMV-specific IgE is a valuable serologic marker for HCMV infection in immunocompromised patients, especially in RTR. Since these patients are undergoing HCMV hyperimmune globulin prophylaxis, an increased HCMV-specific IgG level is not indicative of primary HCMV infection.

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