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Improvement of Serological Diagnosis of Human Cytomegalovirus Infection in Renal Transplant Recipients by Testing for Specific Immunoglobulin E by ELISA

Summary: The kinetics of human cytomegalovirus (HCMV)-specific immunoglobulin E (IgE), M (IgM), A (IgA) and G (IgG) were studied in 421 sera obtained from 19 renal allograft recipients by enzyme-linked immunosorbent assay (ELISA). Cytomegalic inclusion disease (CID) occurred in 11 (57.9%) patients. HCMV infection was diagnosed in all (100%) of these patients by testing for specific IgE. In contrast, increased levels of IgM and IgA class antibody against HCMV were detected in only 45.5% and 18.2% patients suffering from primary or recurrent HCMV infection, respectively. Concerning the time interval between the onset of clinical symptoms and the first positive test, no significant differences in the kinetics of HCMV-specific immunoglobulins E, M, A and G were observed. Elevated specific IgE levels persisted for longer time intervals than the other immunoglobulin classes. As shown by the present study, specific IgE proved to be a more reliable serologic marker than IgM and IgA for the serologic detection of HCMV infection in renal allograft recipients.

Zusammenfassung: *Erweiterung der serologischen Labordiagnose der Zytomegalie bei Nierentransplantationspatienten durch den Nachweis von spezifischem IgE.* Der Nachweis von HCMV-spezifischem IgG, IgA, IgE und IgM wurde in 421 Verlaufsseren von 19 Nierentransplantationspatienten anhand eines Enzymimmunassays (ELISA) untersucht. Eine floride Zytomegalie wurde in 11 (57,9%) Patienten beobachtet. Bei 11 (100%) dieser Patienten gelang die Diagnose einer HCMV-Infektion durch den Nachweis von spezifischem IgE. Spezifische IgA- und IgM-Antikörper wurden nur bei 45,5% bzw. 18,2% der Patienten mit primärer oder sekundärer HCMV-Infektion nachgewiesen. Zwischen dem Auftreten der klinischen Symptome der HCMV-Infektion und dem ersten positiven IgE-, IgG-, IgM- und IgA-Antikörpernachweis wurden keine signifikanten Zeitunterschiede festgestellt. Spezifisches IgE blieb über längere Zeiträume nachweisbar als die anderen Immunglobulinklassen. Die Ergebnisse unserer Studie deuten darauf hin, daß HCMV-spezifisches IgE besser zur serologischen Diagnose der Zytomegalie bei Nierentransplantierten geeignet ist als IgM und IgA.

Introduction

Human cytomegalovirus (HCMV) occurs in more than 50% of patients who receive organ transplants [1] and is one of the most important causes of morbidity in transplant recipients. The frequency of infection and morbidity depends on the degree and type of immunosuppression used for maintenance of the graft and prevention of rejection and the immune status of the patient [1]. In renal allograft recipients, overt cytomegalic inclusion disease (CID) is associated with prolonged fever, diffuse pulmonary infiltrates, retinitis, abnormal liver function, hematologic abnormalities such as leukopenia and thrombocytopenia, and inversion of CD4/CD8 T lymphocyte ratio. Consequently, 20% of renal graft rejections and 25% of all mortality in renal transplant recipients are attributable to HCMV infection [2].

Infection is detected by the isolation of HCMV from clinical specimens, such as urine, bronchoalveolar lavage fluid, induced sputum, and blood buffy coat [3]. Serologic diagnosis of infection is performed mostly by complement fixation (CF) tests and enzyme-linked immunoassays (ELISA), which allow detection of HCMV-specific immunoglobulin of IgG, IgM and IgA classes [4–10]. Even with the most sensitive tests, a proportion of congenitally or neonatally infected infants have been found to produce either no HCMV IgM antibodies or only very small amounts [11–13]. In HIV-seropositive individuals suffering from the acquired immunodeficiency syndrome (AIDS) and in organ transplant recipients, serologic testing for HCMV-specific IgM and IgA antibody often fails to detect HCMV primary infection or recurrence [12, 14, 15]. Recent reports showed that detection of IgE class antibody against HCMV by antibody-capture ELISA improved serologic diagnosis of congenital or neonatal infection [8, 16], HCMV mononucleosis [16] and HCMV primary or recurrent infection in immunocompromised organ transplant patients [16, 17].

In order to investigate the diagnostic benefit of specific IgE antibodies as a serologic marker for HCMV infection, the kinetics of immunoglobulin E were investigated in serial serum samples obtained from renal transplant recipients by an antibody-capture ELISA using commercially available monoclonal anti-IgE coated tubes. The results obtained were compared to those obtained by testing for immunoglobulins A, G and M.

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Patients and Methods

Patient population: A total of 20 patients undergoing renal allograft transplantation during a one-year period from September 1990 to August 1991 at the University Clinics of Frankfurt, Germany, were randomly selected and enrolled in the prospective study. One subject from whom serum specimens were available for only the first six weeks after transplantation was excluded from analysis.

Of the 19 renal transplant recipients (ten male, nine female, mean age 22.2 ± 3.7 years), ten were seronegative for HCMV before renal transplantation. Six of the HCMV-seronegative patients received renal allografts from donors seropositive for HCMV. Intravenous HCMV hyperimmune globulin preparations (Cytoglobin, Tropon, Germany; 300–500 U/kg/week) were administered prophylactically to all six of these patients. Nine renal allograft recipients were HCMV-seropositive before transplantation. Only one of the nine seropositive patients received hyperimmune globulin prophylaxis.

Standard rejection prophylaxis initially comprised prednisolone (1.5 mg/kg/day) plus cyclosporin A supplemented with azathioprine (3 mg/kg/day) until the cyclosporin blood concentration (determinated by TDx Cyclosporin immunoassay, Abbott, Wiesbaden, Germany) reached 200–250 ng/ml. Maintenance treatment entailed the same dose of cyclosporin as above, with prednisolone at 0.25 mg/kg per day. Patients with an acute rejection crisis were given antithymocyte globulin in a dose of 3 mg/kg/day.

Before transplantation, a serum sample was obtained from each of the subjects. After transplantation, all subjects were interviewed for symptoms and examined at least three times per week while they were hospitalized. Subjects were hospitalized for serious rejection episodes (increasing creatinine levels and body weight, reduced diuresis, fever and malaise) or clinical manifestations suspicious for serious infection (interstitial pneumonia, hepatitis, fever of indeterminate localisation).

The patients were routinely monitored weekly during the first six months after transplantation for specific IgG, IgM, IgA and IgE antibody levels by ELISA. A total of 421 serial serum samples were investigated. In parallel, urine samples were taken for viral detection when clinical and/or serological signs of HCMV infection were present.

Antibody-capture ELISA for detection of HCMV IgE antibodies: The test procedure has been described elsewhere [17]. Briefly, lyophilized peroxidase-labeled HCMV antigen (Medac, Hamburg, Germany) was diluted according to the manufacturers' prescriptions in 2.5 ml 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 rinsed with 1 ml washing solution. 50 µl of patient serum were added. After overnight incubation at 4°C, the tubes were washed three times with 1 ml PBS-TT. 50 µl of enzyme-labeled HCMV were added to each tube. After 60 min incubation at room temperature, tubes were again washed three times with 1 ml PBS-TT. Then 50 µl of substrate solution [1 tablet (10 mg) of orthophenylenediamine diluted in 5 ml solvent (Behringwerke, Germany)] 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 2M H_2SO_4 to each tube. Finally, 200 µl of the processed samples were transferred into 96-well microtiter plates and absorbance was read by spectrophotometry (ELISA Processor, Behringwerke) at 492 nm with 650 nm as the reference wavelength. The cut-off level was determined as the mean absorbance plus three standard deviations for 79 sera from HCMV-seropositive renal transplant recipients without clinical signs of HCMV infection and without viruria. The cut-off level was an absorbance of 0.282 ± 0.124.

Indirect ELISA for the detection of HCMV IgG, IgM and IgA antibodies: HCMV IgG, IgM and IgA antibody detection was performed with the commercially available ELISA kit Enzygnost-Zytomegalie® (Behringwerke). All sera were processed as described previously [5,18]. Serological proof of an HCMV infection was defined as a four-fold HCMV-specific IgG titer rise, which was not related to a passive immunisation, or a four-fold IgA titre rise. An IgM titre superior to 1/40 or an IgA titre superior to 1/640 was considered as indicative of an active HCMV infection or reactivation.

Early detection of cytomegalovirus in clinical specimens by cell culture (shell vial culture): Shell vial cultures were performed according to the method of *Schacherer* et al. [15]. Briefly, human foreskin fibroblast cultures were inoculated with clinical specimens (urine). After an incubation period of one to two days, early antigen was detected by a monoclonal antibody (Du Pont, Germany). The reaction was visualized using the immune peroxidase staining method.

Criteria for CID: CID was defined as HCMV infection accompanied by two or more of the following criteria: (i) fever of indeterminate localisation (> 38.5°C, duration > 3 days), (ii) abnormal liver function (SGOT or SGPT > 40 IU/ml), (iii) leukopenia (leucocytes < 3,500/mm^3), (iv) thrombocytopenia (thrombocytes < 100,000/mm^3), (v) interstitial pneumonia and (vi) positive shell vial culture.

Results

Specificity of the IgE Antibody-Capture Assay

To assess the specificity of HCMV IgE detection, 20 serum samples obtained from patients suffering from other acute viral illnesses (herpes simplex virus, Epstein-Barr virus and varicella zoster virus) were investigated. Extinctions under the cut-off level were measured. Serum samples (n = 50) from HCMV-seronegative and healthy seropositive individuals also reacted negative in the IgE ELISA. Throughout the entire study, only one lot of anti-IgE-coated tubes and labelled HCMV antigen was used. Consequently, the intra– and inter-assay variabilities were very low (9.8% and 12.0%). The HCMV IgE concentration of hyperimmune globulin was determined in order to exclude false positive results due to hyperimmune globulin prophylaxis or therapy. The different lots of hyperimmune globulin used in the present study were all HCMV IgE negative.

Clinical Findings

Within 25 to 98 days after transplantation, ten (52.6%) renal allograft recipients (four seronegative and six seropositive) experienced clinical manifestations attributable to HCMV infection (Table 1). Self-limiting disease characterized by mild fever with malaise was observed in two patients 25 and 45 days after renal allograft transplantation. Three subjects had evidence of liver involvement, characterized by elevated transaminases

Table 1: Evaluation of serology for the management of human cytomegalovirus infection in renal transplant recipients (RTR).

Patient group	Hyperimmune globulin	No. of patients with HCMV infection	Positive shell vial culture	Serology			
				IgG[a]	IgM[a]	IgA[a]	IgE[b]
HCMV seronegative RTR (n = 10)							
HCMV positive renal allograft	yes (n = 7)	4	4	0	2	0	4
HCMV negative renal allograft	no (n = 3)	0	0	–	–	–	–
HCMV seropositive RTR (n = 9)							
HCMV positive renal allograft	yes (n = 1)	1	1	0	1	0	1
HCMV negative renal allograft	no (n = 8)	6	6	2	1	4	6
Total	(n = 19)	11	11	2	5	4	11

[a] Seroconversion or fourfold titer increase.
[b] O. D. > cut off.

(34, 35 and 37 days post transplantation) and accompanied in two patients by a watery diarrhea. One seropositive kidney transplant patient had evidence of interstitial pneumonia. HCMV was isolated from urine in this patient concomitantly to pulmonary involvement.

HCMV infection was accompanied by acute rejection episodes in three seronegative and one seropositive renal allograft recipients at 35, 49, 56 and 98 days post transplantation, respectively. Five seronegative and four seropositive renal transplant recipients presented no clinical signs of HCMV infection during the six-month interval of follow-up.

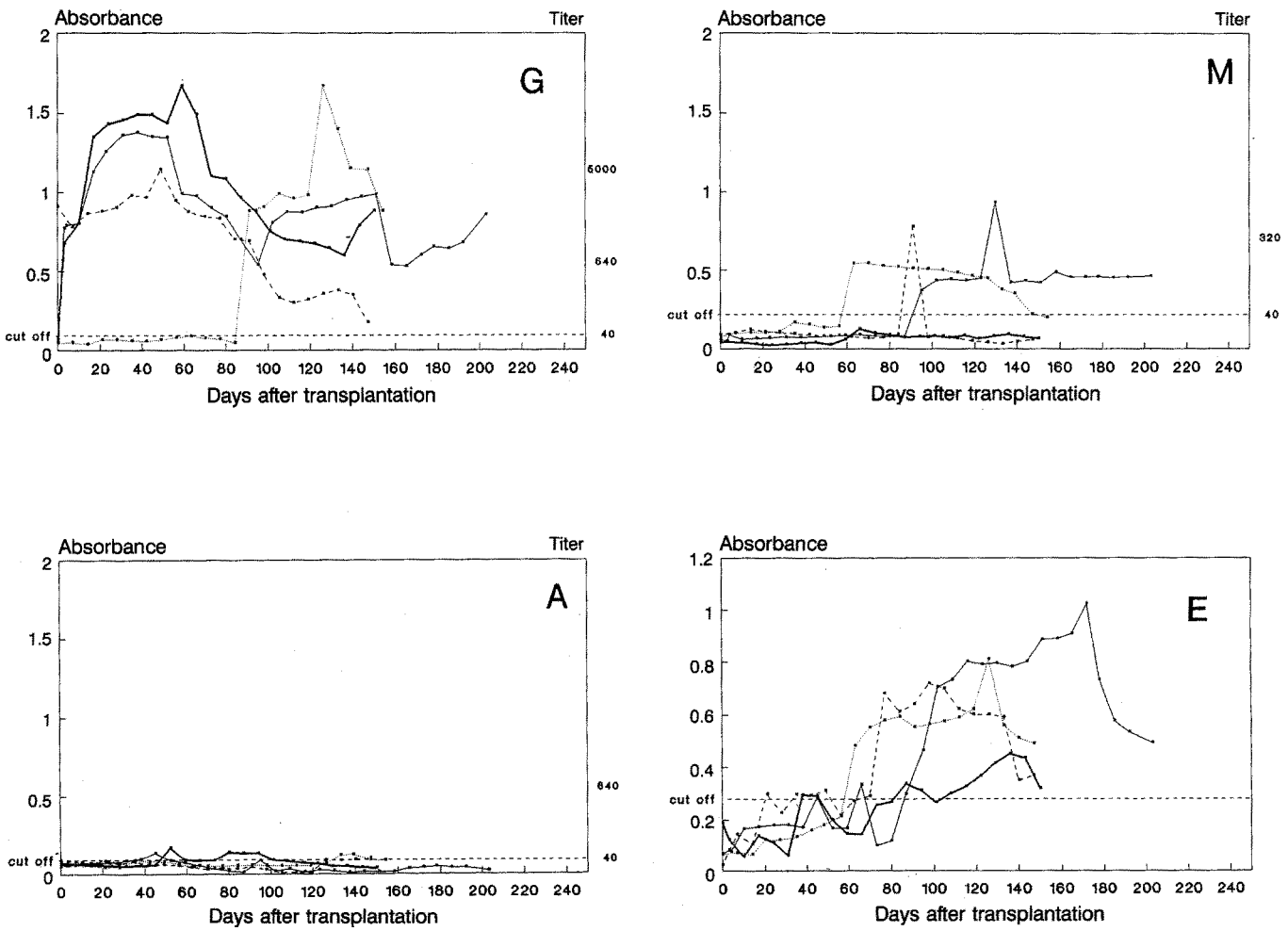

Figure 1: Kinetics of HCMV-specific immunoglobulins G, M, A and E in four renal transplant recipients suffering from primary HCMV infection.

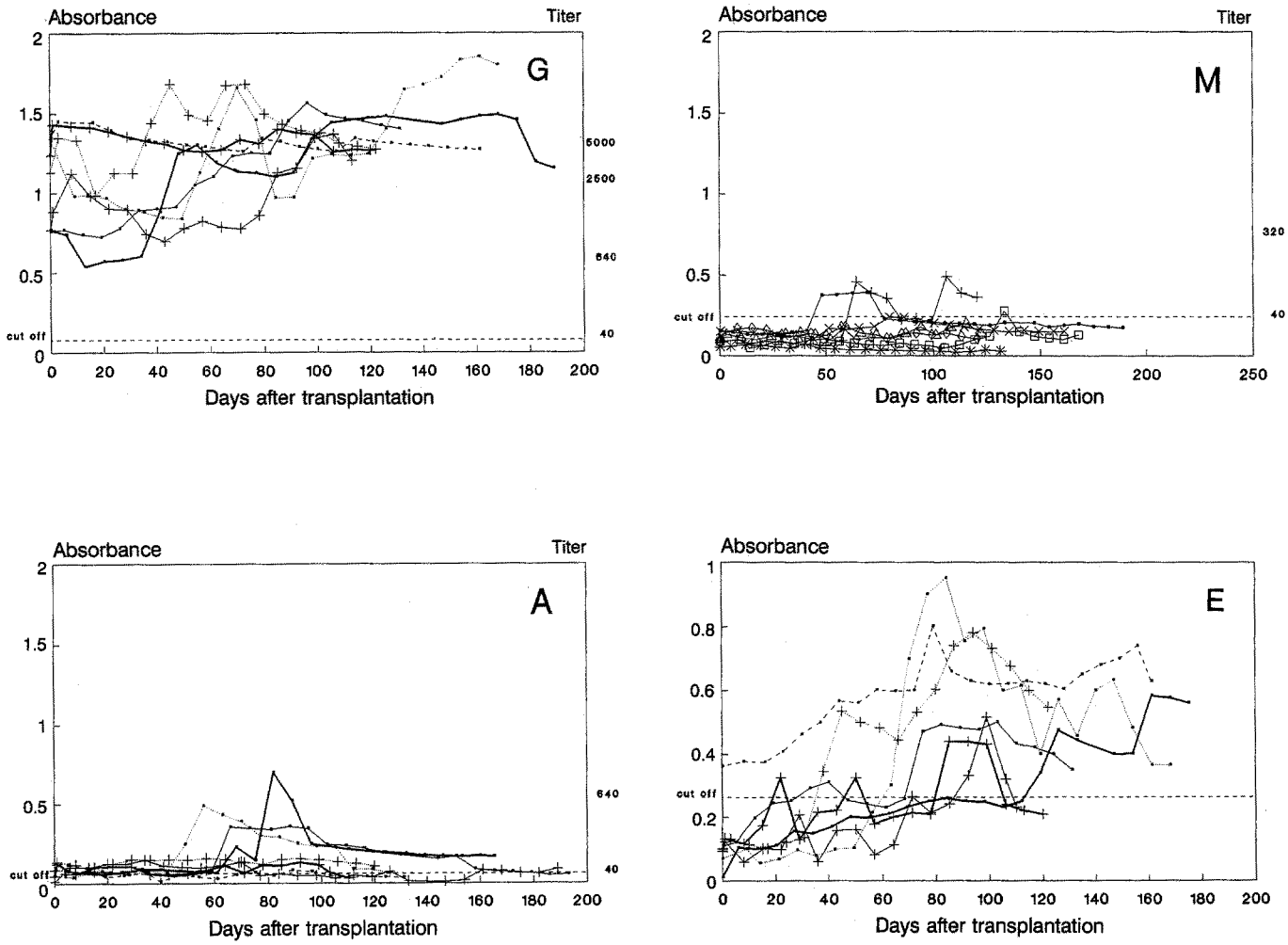

Figure 2: Kinetics of HCMV-specific immunoglobulins G, M, A and E in seven renal transplant recipients suffering from secondary HCMV infection.

HCMV Antibody Response in Renal Transplant Recipients

Serological signs of HCMV infection were present in 11 (57.9%) renal transplant recipients (four seronegative and seven seropositive). Figure 1 shows the antibody response in four renal transplant recipients with primary HCMV infection. All patients were negative for antibodies to HCMV in the four immunoglobulin classes at transplantation. These four patients were treated with prophylactic hyperimmune globulin and presented IgG class antibody titers ranging from 1/640 to 1/5,000 already before primary HCMV infection. Increased IgE class antibody levels to HCMV were detected in all of them. Only one developed specific IgA antibody, while IgM antibody was detected in two. Two patients developed IgM and IgE class antibodies as well. All four seronegative renal allograft recipients showed no titer rise of IgG class antibody attributable to HCMV infection.

The kinetics of specific immunoglobulin class antibodies in seven renal transplant recipients with a secondary HCMV infection are displayed in Figure 2. Elevated IgE class antibody levels were detected in seven seropositive patients, one of whom presented elevated IgE levels already before transplantation. In one patient from whose urine specimen HCMV was isolated, specific IgE antibody was the only serologic marker of HCMV infection detected. In the six other patients with recurrent HCMV infection, detectable IgE was associated with increased titers of IgM and/or IgA.

Transient elevation of IgG class antibody against HCMV not related to passive immunization was observed in two seropositive kidney transplant patients. A total of six seronegative and two seropositive transplant recipients showed no clinical or serological signs related to primary HCMV infection. All of them were found to remain seronegative for HCMV-specific IgE, IgM and IgA.

Timing of Positive Tests

When serologic testing was positive, the mean time interval between the clinical diagnosis of HCMV infection and the first positive test was approximately the same for IgE, IgA and IgM class antibodies. As shown in Figures 1 and 2, when specific IgE was present, it could be detected from 14 to more than 161 days (mean: 83.4 days) post infection. No significant difference in the persistence of

IgE class antibody was observed between primary and secondary HCMV infection (92.8 days, range: 77–130 days vs 83.4 days, range: 14–161 days). Elevated levels of IgA and IgM were detectable for significantly shorter periods of time (IgM: 39.8 days, range: 14–63 days; IgA: 40.5 days, range: 18–82 days) than IgE.

Discussion

The aim of this study was to evaluate the role of HCMV IgE antibody as a supplementary serologic marker for HCMV infection in renal transplant recipients. 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 [7–9,15–17]. The direct ELISA using enzyme-labeled antigen is not impaired by rheumatoid factor or competitive IgG antibodies [18,19]. In the present study, unlabeled antigen was not used as a control, since in a previous report using the same technique, negative extinctions were measured in all sera tested when they were incubated in the presence of control antigen [8]. Furthermore, HCMV IgE is not detected in HCMV-seronegative or -seropositive healthy individuals [17]. According to these findings, in the present study, HCMV-specific IgE could not be detected in asymptomatic HCMV-seropositive and -seronegative renal transplant recipients. HCMV infection occurred in 11 (57.9%) of 19 renal transplant recipients (Table 1). HCMV-specific IgE proved to be a more reliable serologic marker for primary or secondary infection in renal allograft recipients since it was detected more frequently (100%) than IgM (45.5%), IgA (36.4%) and IgG (18.2%) in patients suffering from HCMV infection. Elevated IgE levels were present in all patients with clinical symptoms attributable to HCMV infection (CID) and/or from whom HCMV was isolated in the urine by the shell vial culture method. Moreover, as shown by our results, IgE class antibody against HCMV was detectable over longer time intervals than IgA and IgM. For all these reasons, the detection of specific IgE is the most suitable serologic assay for diagnosis of recent HCMV infection.

In agreement with the results obtained from previous studies [16], increased IgE levels can be observed in primary HCMV infection. In contrast to *Van Loon* et al. [16], who detected HCMV IgE in only 9% of patients suffering from recurrent infection, a 100% HCMV IgE seropositivity rate was found in renal transplant recipients suffering from HCMV recurrence. This was realized by optimizing our test system through a higher antigen concentration and a reduction of the cut-off level.

Similar to IgA class antibody against HCMV [4,5,9], specific IgE is present in immunocompromised subjects with HCMV reinfection or reactivation [16]. Nevertheless, in our study IgE represented a more reliable marker than IgA for serological diagnosis of HCMV recurrence, since it was detected in seven seropositive renal transplant recipients while only three patients showed elevated IgA levels.

Present data confirm that HCMV IgE is a reliable marker for HCMV seroconversion in seronegative renal allograft recipients with HCMV infection. Since these patients receive HCMV hyperimmune globulin prophylaxis, an increased HCMV IgG level is not indicative of primary HCMV infection.

As shown by our results, IgE class antibody against HCMV may be considered a specific serologic marker for HCMV infection, since it was associated with virus isolation consistently.

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Book Review

G. P. Bodey (ed.)
Candidiasis: Pathogenesis, Diagnosis and Treatment
432 pages, 70 figures
Raven Press, New York 1992
Price: $ 119.--

Candidiasis is an increasingly important infection. Decades ago disseminated candidiasis was very rare. Now it has emerged as a significant medical problem. What are the reasons for the dramatic change? Patients with debilitating illnesses and immunocompromised patients who formely died of bacterial infections are now surviving due to the availability of more potent antibacterial agents.

Epidemiologically, *Candida* spp. are the fifth most common primary blood stream organisms and the seventh most common pathogens to cause nosocomial infections.

Candida infection of the oropharynx, esophagus and vagina is often the first manifestation of AIDS and recurrent *Candida* infections are common in this patient population.

The diagnosis of deeply invasive candidiasis remains difficult, because the clinical manifestations are not specific. Laboratory methods have been improved during the past 20 years. Nevertheless, deeply invasive candidiasis is difficult to diagnose and is the cause of substantial morbidity and mortality in hopitalized patients.

Detection of *Candida* spp. in blood cultures is one of the most substantial advances in diagnostic procedures. But early blood culture detection techniques often did not recover *Candida* spp. (Disparity between postmortem tissue findings and the paucity of premortem positive blood cultures).

In this book the various biochemically and molecularly defined investigational markers for the detection of invasive candidiasis are summarized in a special table. The diagnostic value of anti-*Candida* antibodies, antigen cell wall mannan and uncharacteristic agglutination antigens is critically reviewed. Various cytoplasmatic antigens may often be another marker for detection of invasive candidiasis: *Candida* enolase antigen, anti-*Candida* enolase antibody HSP-90-related *Candida* antigen and different *Candida* metabolites and cell-wall components.

Bedside patient evaluation, blood cultures, diagnostic imaging and biopsies (cultures) are the standard clinical and laboratory tools in diagnostic and therapeutic monitoring and invasive candidiasis. But all these methods lack sensitivity in the early recognition of infection and are imprecise as markers of complete eradication of infection. All available diagnostic tools are for the moment of limited usefulness. No serological test for detection of *Candida* antigen has proved to be universally reliable. Therefore we hope for new approaches now under investigation.

The chapters addressing radiological features and cutaneous manifestations of *Candida* infection are written very knowledgeably. The figures are highly instructive.

The critical review of antifungal agents by *P. Bodey* is very interesting, because of the different therapeutic concepts in the United States and Europe.

The outlook for new developments of a variety of amphotericin B lipid complexes or liposomes currently at varying stages of clinical investigation is fascinating. They include:

amphotericin B (A) lipid complex (ABLC)
A-colloidal dispersion (ABCD)
A-unilamellar liposomal preparation.

Very important are the critical remarks of the authors at Baylor College of Medicine (Houston, Texas). The diagnosis of *Candida* endocarditis is based on clinical and laboratory criteria. Clinical signs and symptoms of *Candida* endocarditis rarely distinguish it from bacterial endocarditis. Presentation with a major arterial embolus immediately increases the likelihood of fungal endocarditis. Blood cultures are frequently positive in patients with *Candida* endocarditis (80–90%). Echocardiography and serological tests could be very helpful.

This is the second edition of this book. It can be recommended as an outstanding reference book – one of the best and most critical documentations on the subject.

We are grateful to all the authors who try to transmit their extensive experience to the "bed-side clinician."

T. Wegmann
St. Gallen, Switzerland