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Early diagnosis of CMV infection by detection of pp65 antigen in 91 renal transplant recipients

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Abstract We evaluated how accurately a CMU antigenemia test correlated with classical CMU infection markers. We studied 91 kidney transplant recipients from February 1991 to June 1992. Antigenemia (pp65 antigen) was positive in 100% of cases of primary infection and in 70% of cases of reactivation and/or reinfection. Furthermore, antigenemia detected more infections (71%) than viremia (16%). The antigenemia test proved to be highly specific: it remained consistently negative in 22 seronegative patients, as well as in 19 of 20 seropositive recipients without recurrent infection. pp65 Antigen in the polymorphonuclear leukocytes was detected earlier than, or simultaneously with, virus culture in 78% of cases and became positive before serologic tests of primary and secondary infection in nearly 90% of cases. Most importantly, the antigenemia test detected all of the symptomatic cases.

Key words Kidney transplantation, cytomegalovirus · pp65 antigen, CMV

Introduction

Cytomegalovirus (CMV) is reported to occur in about 40%–70% of all renal transplant recipients [10, 12]. Most infections develop within the first 4 months after transplantation [8]. CMV may lead to potentially life-threatening disease. Furthermore, it may directly or indirectly impair graft function [15]. The latter setting is not always easy to differentiate from rejection. However, the distinction between the two conditions is very important, as their therapeutic approach is completely different [17]. Thus, clinicians need a marker of CMV infection that can provide them with an early and rapid diagnosis. CMV antigenemia, which detects a viral matrix protein (pp65) inside the polymorphonuclear leukocytes, is one of the newly proposed tests that may be more suitable for detecting active infection and symptoms than classical virological tests [2]. In a prospective study of 91 consecutive kidney recipients followed for at least 6 months after transplantation, we compared this test to viral isolation and serology in relation to clinical manifestations and sought to determine whether it could be applied to select a group of high-risk patients who would benefit rapidly from ganciclovir, a very effective anti-CMV drug [6, 7, 11].

Materials and methods

Patients

Ninety-one consecutive kidney recipients transplanted between February 1991 and June 1992 were studied. They included 57 males with a mean age of 36 years (range 4–66 years) and 34 females with a mean age of 37.5 years (range 7–59 years). The graft originated from a cadaveric donor in 76 cases and from a living donor in 15 cases. The graft was the first one in 82 cases and the second in 9 cases. Pretransplant CMV serology was determined in each donor/recipient pair. In the 45 patients exclusively followed at our outpatient clinic, CMV serology, virus isolation (in blood, urine, and saliva), and antigene-

Table 1 Incidence of pp65 antigenemia in cases of CMV infection

Category	pp65	Blood	Urine	Virus[a]	IgM antibodies	High CF[b]	CMV disease	Pneumonia
a	0/22 0%	0/22 0%	0/22 0%	0/22 0%	0/22 0%	0/22 0%	0/22 0%	0/22 0%
p	5/5 100%	3/5 60%	5/5 100%	5/5 100%	5/5 100%	5/5 100%	4/5 80%	2/5 40%
e	1/20 5%	0/20 0%	0/20 0%	0/20 0%	0/20 0%	0/20 0%	0/20 0%	0/20 0%
r	30/44 68%	5/44 11%	34/44 77%	36/44 82%	15/44 34%	41/44 93%	2/44 5%	0/44 0%

[a] Positive culture in urine, blood, and saliva
[b] Fourfold increase in CF antibodies or high titer ≥ 1/128

mia were performed biweekly for the first 90 days and afterwards weekly up to 6 months. In the 46 other patients, the same tests were performed biweekly for the first 36 days; afterwards, only serology was available once a week. Therefore, for this second cohort of recipients, the comparisons between pp65 antigen and the other markers of infection were made for the first 36 days.

The results of pp65 antigen were never transmitted to the clinicians until the end of the study.

Antigenemia

Antigenemia was assayed with the Clonab monoclonal antibody that recognizes a protein of 65 KD (pp 65) from the viral lower matrix [4, 9, 13, 16]. In brief, after blood separation with dextran, the buffy coat was cytocentrifuged (Cytospin 3, Shandon) in order to get a preparation of 50,000 leukocytes per microscopic slide. Using the Clonab monoclonal antibody mixture of C10, C11 (Biotest, Dreieich, Germany) as a first layer, the immunoenzymatic reaction was completed using the APAAP method [1]. The result was expressed as the total positive number of leukocytes per 50,000 cells, counted with light microscopy (× 40).

CMV isolation

Samples of urine and saliva were filtrated on a 0.45-μm single use filter unit (Minisart, Sartorius, Germany); 0.2 ml of the samples was inoculated in duplicate on human embryonic lung cells (MRC5 cell line, Biomérieux, Lyon, France), centrifuged at 3000 rpm for 45 min on macroplates (Nunclon, Roskilde, Denmark), and incubated at 37 °C. After 48 h they were fixed in a solution of 90% acetone and 10% distilled water at −20 °C; replication of CMV was detected by immunoperoxidase staining with a monoclonal antibody (clone E13) to CMV immediate early antigen (Biosoft, Paris, France). Blood buffy coat was processed identically, without the filtration step. The same samples were also cultured on tubes to detect characteristic cytopathic effect (CPE).

Serologic methods

Sera were titrated quantitatively for anti-CMV IgM and IgG by ELISA (α method; Behring, Marburg, Germany) and for complement fixing (CF) by anti-CMV antibodies on microplates (Virion antigen).

Definitions

CMV infection

Primary infection was defined as seroconversion in IgM, IgG, or CF antibodies with virus isolation in urine and saliva. In seropositive patients, reactivation and/or reinfection was defined either by a fourfold titer increase in CF and IgG antibodies with or without IgM detection and/or by CMV isolation in urine and saliva.

CMV disease

CMV disease was defined as CMV infection with fever unexplained by another cause for at least 48 h, with or without arthralgia, leukopenia, or thrombocytopenia.

CMV pneumopathy

Pneumopathy was defined as CMV disease with interstitial pneumonitis and/or with positive virus isolation in bronchoalveolor lavage (BAL).

Immunosuppressive drugs

In all recipients, postoperative therapy included rabbit antithymocyte globulins (RATG, Fresenius, Germany) associated with azathioprine, 1 mg/kg per day, cyclosporin, starting at 3 mg/kg per day, progressively increasing to 5–10 mg/kg per day, and thereafter adjusted according to blood cyclosporin and serum creatinine levels, and prednisolone, started at a dose of 0.5 mg/kg per day, progressively tapered to 0.1 mg/kg per day after 9 months. Acute rejection was treated with a 10-day course of OKT3, 5 mg/day (Orthoclone, Cilag, Switzerland).

None of the patients received hyperimmune anti-CMV globulin or high-dose oral acyclovir as prophylaxis.

Virological groups

Category **a** included seronegative recipients before transplantation who remained seronegative after transplantation. Category **p** included seronegative recipients before transplantation who seroconverted after transplantation. Category **e** included seropositive recipients before transplantation who remained seropositive after transplantation, without reactivation and/or reinfection. Category **r** included seropositive recipients before transplantation who had reactivation and/or reinfection after transplantation.

Table 2 Day of detection of pp65 antigenemia compared to other markers of CMV infection (*CF* complement fixation antibodies)

Name	Category	pp65	Virus[a]	IgM	CF
bo	p	52	52	64	[b]
br	p	55	81	85	[b]
co	p	34	10	45	[b]
ru	p	44	71	47	[b]
to	p	93	97	63	[b]
an	r	18	33	[b]	[b]
ca	r	[b]	16	[b]	[b]
es	r	[b]	62	[b]	[b]
ab	r	[b]	55	[b]	100
al	r	29	29	[b]	75
an	r	26	28	[b]	76
ap	r	18	25	[b]	53
be	r	55	59	[b]	56
bn	r	25	32	[b]	69
bi	r	34	51	[b]	48
cl	r	15	22	[b]	109
co	r	43	53	[b]	99
fo	r	[b]	77	[b]	97
ga	r	[b]	43	[b]	106
gi	r	34	92	[b]	76
ik	r	[b]	143	[b]	63
li	r	37	37	[b]	44
lo	r	49	42	[b]	48
mi	r	21	29	[b]	56
mo	r	[b]	132	[b]	90
ro	r	30	10	[b]	32
ru	r	39	58	[b]	44
tr	r	60	68	[b]	68
vo	r	25	34	[b]	60
zo	r	35	35	[b]	79
ar	r	222	194	44	44
bo	r	21	21	35	60
de	r	163	69	58	58
di	r	31	[b]	33	[b]
ek	r	20	8	72	5
he	r	49	49	60	60
ki	r	[b]	43	39	32
ma	r	[b]	37	37	48
mi	r	52	[b]	59	59
pe	r	31	[b]	52	52
pu	r	[b]	62	28	48
ru	r	31	34	58	58
sa	r	47	33	47	47
si	r	23	23	31	72
so	r	23	23	40	70

[a] Positive culture in urine, blood, and saliva
[b] Consistently negative

Donor/recipient categories:

D − /R − : seronegative donor and seronegative recipient

D + /R − : seropositive donor and seronegative recipient

D − /R + : seronegative donor and seropositive recipient

D + /R + : seropositive donor and seropositive recipient.

Results

Correlation between antigenemia (pp65) and other CMV infection markers (Table 1)

Twenty-seven recipients (categories **a** and **p**) were seronegative before transplantation. Of the 22 patients who remained seronegative (category **a**), none was found positive for antigenemia; of the 5 recipients with primary infection (category p), CMV was isolated from urine in 5 and from blood in 3 and pp65 antigen was positive in all (100%).

There were 64 recipients who were seropositive before transplantation (categories **e** + **r**). Of the 20 patients with no markers of reactivation or reinfection (category **e**), only 1 (5%) had a positive result for pp65 antigen. Of the 44 recipients with recurrent infection (category **r**), 3 reactivated exclusively by viral excretion, and pp65 antigen was positive in 1 of them (33%); 4 patients reactivated exclusively by serology and had negative results for pp65 antigen. Twenty two patients reactivated by both serology and viral isolation; 4 of them (18%) had a positive result for blood culture and 17 (77%) were positive for the antigenemia test. Fifteen patients reactivated with IgM detection became reinfected, 9 had a positive CMV culture, and 12 had positive results for pp65 antigen.

The sensitivity (true-positive/total positive) and specificity (true-negative/total negative) of pp65 were 35/49, or 71.43%, and 41/42, or 97.62%, respectively. With blood culture they were 8/49, or 16.33%, and 42/42, or 100%, respectively.

Temporal relationship between antigenemia and other markers of CMV infection (Table 2)

PP65 compared to virus cultures

Antigenemia was detected before virus culture in 16 out of 32 cases (50%), simultaneously with virus culture in 9 out of 32 cases (28%), and after virus culture in 7 out of 32 cases (22%).

PP65 compared to IgM antibody detection

Antigenemia was detected before IgM antibodies in 13 out of 17 cases (76%), simultaneously with IgM antibodies in 3 out of 17 cases (18%), and after IgM antibodies in 1 out of 17 cases (6%).

PP65 compared to complement fixing (CF) antibody detection

Antigenemia was detected before CF antibodies in 28 out of 33 cases (85%), simultaneously with IgM antibodies in 2 out of 33 cases (6%), and after CF antibodies in 3 out of 33 cases (9%).

Table 3 Early diagnosis of six symptomatic patients by pp65 antigenemia (*Arth* arthralgia, *Pneu* pneumonia, *Leuk* leukopenia, *Thrombo* thrombocytopenia)

Name	Active infection	Day	Virus[a]	Day	pp65	Day	Fever	Arthr	Pneu (BAL [+])	Leuko	Thromb
bo	Primary	52	+	52	+	52	+	(−)	(−)	+	(−)
br	Primary	87	+	55	+	52	+	(−)	+	(−)	(−)
ru	Primary	71	+	33	+	47	+	(−)	(−)	+	+
to	Primary	95	+	93	+	93	+	(−)	+	(−)	(−)
ap	Reactivation	25	+	18	+	32	+	(+)	(−)	+	(−)
bu	Reactivation	21	+	21	+	21	+	(−)	(−)	+	+

[a] Positive culture in urine, blood, and saliva

Table 4 Monitoring of pp65 antigenemia in renal transplant recipients

D/R	pp65	Blood culture	Urine culture	Virus culture[a]	IgM	High FC	CMV disease	Pneumonia	Virological follow-up
D − /R −	0/18 0%	0/18 0%	0/18 0%	0/18 0%	0/18 0%	0/18 0%	0/18 0%	0/18 0%	Only symptomatic patients
D + /R −	5/9 56%	3/9 33%	5/9 56%	5/9 56%	5/9 56%	5/9 56%	4/9 44%	2/9 22%	Biweekly for 3 months
D − /R +	7/20 35%	1/20 5%	10/20 50%	10/20 50%	6/20 30%	13/20 65%	1/20 5%	0/20 0%	Only symptomatic patients
D + /R +	24/44 55%	4/44 9%	24/44 55%	27/44 61%	9/44 20%	27/44 61%	1/44 2.2%	0/44 0%	Only symptomatic patients

[a] Positive culture in urine, blood, and saliva

Correlation between antigenemia and CMV disease

Four patients with primary infection and two patients with recurrent infection had CMV disease. All of them suffered from fever, two from pneumonia, and one from arthralgia. Leukopenia occurred in four cases and thrombocytopenia in two cases. All of them were positive for pp65 antigen (Table 3).

Correlation between antigenemia (pp65) and donor/recipient serostatus (Table 4)

No CMV infection occurred in any of the 18 seronegative recipients grafted with kidneys from seronegative donors and all of the markers of infection remained negative.

Of the nine seronegative patients grafted with an organ from a seropositive donor, five experienced primary infection, all five were positive with pp65 antigen, and three with blood culture.

Of the 20 seropositive patients grafted with a kidney from a seronegative donor, 13 experienced reactivation and/or reinfection, 7 were positive for pp65 antigen, and 1 for blood culture.

In the last caterogy of 44 seropositive patients grafted with an organ from a seropositive donor, 27 reactivated or were reinfected; 24 were detected by pp65 antigen and 4 by blood culture.

Quantitation of pp65

In cases of primary infection, the number of positive cells ranged from 10 to 82/50,000 cells. In cases of reactivation, the range was very wide: 1–282 positive cells/50,000 cells.

Discussion

CMV infection is a very frequent event that interferes with renal transplantation in about 40%–70% of all cases [8, 10, 12], a fact confirmed by our series with 54% of infections. The rates of morbidity and mortality associated with CMV infection and the efficacy of ganciclovir has stressed the need for more rapid tests for diagnosis, prognosis, and therapeutic purposes.

In a prospective study of 91 renal transplant recipients, we assessed how CMV antigenemia correlated with classical virological tests and clinical data. In our experience, the sensitivity of the test to detect CMV infection differed in primary infection (100%) and in reactivation (68%; Table 1). If we only consider the cases with positive CMV isolation (primary or recurrent infection), the sensitivity of pp65 to detect those cases is 87.7%.

The specificity of the test was very high (98%). The only conflicting result was from a recipient treated for acute rejection who was found positive for antigenemia without any other positive marker of CMV infection. We decided to record this antigenemia result as a false-positive although this patient became infected by CMV after

6 months of observation (Table 1). Our data on sensitivity and specificity corroborate the figures of 89% for sensitivity and 93% for specificity previously reported [2, 18]. As in previous studies [3, 5, 16], antigenemia confirms its higher sensitivity (71.4%) to detect CMV infection when compared to blood culture (16%).

Antigenemia demonstrated its best sensitivity in the detection of symptomatic patients since all of them (6/6) were positive for the test; pp65 antigen was detected, on the average, 20 days before viral excretion in four cases and simultaneously in two cases; pp65 was detected 14 days before fever in two cases, simultaneously in three cases, and 3 days after fever in one case (Table 3).

Going through the different D/R categories (Table 4), we note that no seroconversion occurred in the D −/R − category, showing the importance of the donor CMV serostatus for a seronegative recipient. All of these patients also remained negative for the antigenemia test. In contrast, in the D +/R − group, 56% of the recipients experienced primary infection, and all of them were detected by antigenemia. They were all symptomatic except for one, who incidently also had the lowest number of positive cells for the antigenemia test. All of the other symptomatic recipients had over 50 positive cells. This excellent correlation with primary infection would make one inclined to use the antigenemia test on a routine basis for monitoring recipients at high risk of primary infection, essentially seronegative patients receiving a kidney from a positive donor (D +/R −).

In cases of recurrent infection (D +/R + D −/R +), antigenemia was positive in 70% of the cases, while there were only two symptomatic patients (4.5%). This is in line with previously reported observations [10, 14]. Thus, patients with secondary infection clearly do not benefit from this test. Therefore, in addition to classical viral tests, we advocate a routine, biweekly screening with antigenemia in the D +/R- group within the first 3 months after transplantation. In the three other categories (D −/R −, D −/R +, D +/R +), antigenemia testing, together with the other infection markers at the onset of symptoms of infection or rejection, may prove sufficient for the management of these patients. In our hands, this test is often the first one to become positive, providing an early diagnosis for CMV infection. Moreover, the manipulation time does not exceed 7 h, compared to the 72-h minimum for viral isolation, which makes it more valuable for rapid clinical measures.

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