

## Antibodies against cytomegalovirus-induced early antigens (CMV-EA) in immunosuppressed renal-allograft recipients

T. H. THE,\* H. K. ANDERSEN,† E. S. SPENCER‡ & G. KLEIN§ \* *Clinical Immunology Unit, Department of Internal Medicine, Groningen, The Netherlands*, † *Institute of Medical Microbiology, University of Aarhus*, ‡ *First Medical University Clinic, Kommunehospital, Aarhus, Denmark* and § *Department of Tumorbiology, Karolinska Institute, Stockholm, Sweden*

(Received 20 January 1977)

### SUMMARY

Antibodies against cytomegalovirus-specific early antigens (CMV-EA) were followed in sera, obtained from fifteen immunosuppressed renal-allograft recipients. Eight patients (sixty-two sera) showed seroconversion 47–137 days post-transplantation. Five patients (forty sera) with CMV antibodies at the moment of renal implantation all showed CMV-EA antibody rises. Two patients remained seronegative until 4 years after transplantation. Thus, in immunosuppressed patients, antibodies against CMV-EA remained at high titres during many years (4–8 years) after transplantation as distinct from the apparently transient nature in acutely infected previously healthy adults with CMV mononucleosis or post-perfusion syndrome.

These results support the view that CMV-EA antibodies reflect an active viral proliferation in the host.

### INTRODUCTION

Cytomegalovirus (CMV), a member of the Herpes virus group, is for several reasons an interesting infectious agent in man. Many years ago, its role in causing congenital cytomegalic disease with multiple birth defects was established (Weller & Hanshaw, 1962; Krech *et al.*, 1971). In this connection CMV has been incriminated as a major cause of mental retardation in childhood (Stern *et al.*, 1969). In adolescents or young adults CMV causes an infectious mononucleosis-like disease (Kääriäinen *et al.*, 1966). In immunologically compromised patients with malignant tumours, especially leukaemias and lymphomas, CMV is of major concern in causing tissue injury and death (Duwall *et al.*, 1966). This also applies for immunosuppressed kidney-allograft recipients (Craighead *et al.*, 1967). However, in the normal human population, most CMV infections are subclinical or very mild and once introduced, CMV persists for years, if not for the lifetime of the host (reviewed by Weller, 1971).

Previous studies with regard to CMV-induced antigens in tissue cultures, have shown production of two different types of nuclear antigens. The first, CMV early antigens (CMV-EA), are produced in the absence of viral-DNA synthesis (Ara C block), while the second, CMV late antigens (CMV-LA), are produced in cultures, where viral-DNA synthesis in infected cells can proceed to production of viral particles (The *et al.*, 1974). Using an indirect-immunofluorescence method, different serological reaction patterns were recognized in relation to time after primary CMV infection. Sera from normal donors, containing complement fixing (CF) antibodies, indicating chronic CMV infections, showed antibodies against CMV-LA only. Antibodies against CMV-EA were detected temporarily in sera from patients until about 3 months after an acute CMV infection. Serially collected serum samples from patients with CMV mononucleosis showed that production of CMV-EA antibodies reached peak titre values 4–9 weeks after onset of symptoms. Thereafter CMV-EA antibodies declined to low values after 3 months.

Correspondence: Dr T. H. The, Clinical Immunology Unit, Department of Internal Medicine, University Hospital, Oostersingel 59, Groningen, The Netherlands.

In contrast CMV-LA and CF antibodies remained in high titres for many years. Antibodies against CMV-EA are therefore of considerable value for diagnosing a primary or acute CMV infection (The *et al.*, 1974).

The immunosuppressive therapy used in recipients of renal allografts predisposes to active infection from latent or newly introduced herpes group-viruses especially CMV (Craighead *et al.*, 1967; Andersen & Spencer, 1969). Therefore CMV-EA antibodies were followed in patients with primary post-transplant CMV infection and in patients with reactivated CMV infection after transplantation. The results with 116 sera from fifteen patients covering observation periods from 8 months to 8 years will be presented and discussed.

## MATERIALS AND METHODS

*Patients' sera.* Sera from renal allograft recipients were collected in connection with earlier studies (Andersen & Spencer 1969; Spencer, 1974). Three groups of patients were selected on results of the CMV complement-fixation (CF) test. Group I, (Table 1 no. 1-8), eight seronegative cases with seroconversion during the first months after transplantation. Group II, (Table 1 no. 9-13), five seropositive cases with rises in CMV-antibody titres after transplantation. Group III, (Table 1, no. 14-15), two patients who remained seronegative for 2-3 years.

*Immunosuppressive treatment.* Azathioprine, 2-4 mg/kg body weight, was used from or before the day of transplantation in all cases. Prednisone, 300-150 mg/day, was started at the first sign of a rejection crisis and thereafter slowly reduced to a maintenance dose of 10-20 mg/day, unless renewed signs of rejection appeared, whereupon the dose was again increased. Further details are described in a previous paper (Andersen & Spencer, 1969).

*Serological tests.* The CMV-CF test was performed with CMV strain AD 169 antigen in Cooke microtitre system using 4 u of antigen, 1.5 u of complement and 8 u of haemolysin. Tests for neutralizing (NT) antibodies were performed in a plaque-NT system using strain AD 169 as described previously (Andersen, 1971).

Antibodies against CMV induced early (EA) and late (LA) antigens titrated as described in a previous study (The *et al.*, 1974). In short, indirect immunofluorescence method was applied on target cells containing CMV-EA obtained by infection of human embryonic fibroblasts treated with arabinoside (Ara C) in a dose sufficient to prevent DNA synthesis.

Infected, but otherwise untreated cells that were allowed to proceed to the synthesis of complete viral particle were used as targets containing CMV-LA.

## RESULTS

The results with 116 sera from fifteen patients are summarized in Table 1. The patients with a primary post-transplant CMV infection, no. 1-8, represented by sixty-two sera, showed seroconversion in CF and LA antibodies from day 47 to day 137 post-transplantation. Antibodies to CMV-EA rose in all patients. Comparing the titres of the four antibodies in the individual patients it can be seen that the production of CMV-EA antibodies differed from the three other ones. The EA antibodies lagged behind CF and LA antibodies, but appeared before NT antibodies. Patients with CMV antibodies at the moment of renal transplantation, no. 9-13, were represented by forty sera. CMV-EA and -LA antibodies showed further increases in all cases but one, patient 13, who had high levels of CMV antibodies already in the first serum studied. CMV-EA antibodies were maintained at high titres in almost all patients, patients no. 2 and 13 being followed for 4 and 8 years respectively. Two control patients, no. 14 and 15, remained seronegative for all four CMV antibodies until 2 years after transplantation.

## DISCUSSION

The present study showed that CMV-EA antibodies increased and remained at high titres for many years in patients chronically treated with immunosuppressive drugs to ensure acceptance of renal allografts. This apparent persistence in CMV-EA-antibody production is different from the apparently transient nature of CMV-EA antibodies in acutely infected previously healthy adults with CMV mononucleosis or a post-perfusion syndrome (The *et al.*, 1974). These findings lead us to the conclusion that CMV-EA antibodies reflect an active viral proliferation in the host because overwhelming data are available that immunosuppression results in patients' incapability to terminate CMV proliferation (Craighead *et al.*, 1967; Andersen & Spencer, 1969).

TABLE 1. Antibodies against CMV antigens in 116 sera from fifteen renal allograft recipients

Patient no. Age M/F	Time-post-transplant	CMV-antibody titres			
		EA	LA	CF	NT
1	10d	< 40	< 40	< 4	< 4
27 M	3,5,6,9 m	≥ 3200	≥ 3200	54	> 10
2	-22 d	< 40	< 40	< 4	< 2
25 M	48 d	400	≥ 3200	32	< 2
	3,4,8,15 m,4 y	≥ 3200	≥ 3200	128	20-150
3	-1, 22 d	40	40	4	2
24 F	61 d	40	≥ 3200	32	≤ 2
	3,4 m,2,3,5 y	≥ 3200	≥ 3200	32	64-128
4	5,45,96 d	< 40	< 40	< 4	< 2
12 F	4 m,2,3 y	≥ 3200	≥ 3200	128	2-> 10
	4 y	400	400	64	n.d.
5	-4,24,45 d	< 40	< 40	< 4	≤ 2
19 F	5,10,15 m	≥ 3200	≥ 3200	64	> 10
6	30 d	< 40	< 40	< 4	< 2
27 F	54 d	40	800	8	< 2
	3 m	800	1600	128	≤ 2
	4 m	1600	≥ 3200	256	n.d.
	5,7 m,2 y	≥ 3200	3200	256	64-256
7	2,33 d	< 40	< 40	< 4	< 2
17 F	55 d	40	400	32	16
	3,4,5 m	1600	≥ 3200	256	8-128
	8 m,1,2,3 y	≥ 3200	≥ 3200	256	128
8	-2 d, 0 d, 3,15 d	< 40	< 40	< 4	< 2
28 M	47 d	< 40	1600	64	≥ 2
	7, 11 m	1600	≥ 3200	256	≥ 128
	18 m, 2 y	≥ 3200	≥ 3200	256	n.d.
9	-25 d	< 40	800	4	32
29 M	-21 d	1600	≥ 3200	64	n.d.
	-4 d	≥ 3200	≥ 3200	64	128
	19,28,35,42 d,9,20 m,4 y	800	≥ 3200	64	64-16
10	3,38 d	400	400	16	20
25 F	3, 4 m	1600	1600	32	20
	7,11,14 m, 6 y	≥ 3200	≥ 3200	64	100
11	-1,15 d	40	400	64	64
41 F	71 d, 15 m	800	≥ 3200	128	64
	18 m, 2 y	≥ 3200	≥ 3200	128	128
12	-2,28 d	800	≥ 3200	128	≥ 128
47 F	4 m	≥ 3200	≥ 3200	128	256
	8 m	1600	≥ 3200	128	128
13	12,45,54 d,2,3 m,1,2, 3 y	1600	≥ 3200	32	32
32 F	4,7,8 y	≥ 3200	≥ 3200	64	32
14	-18, 23 dy,				
46 F	2,3,6,11 m,2 y	< 40	< 40	< 4	< 10
15	5,22,53 d	< 40	< 40	< 4	< 10
42 F	3, 5,10 m,2 y				

EA = CMV early antigens; LA = CMV late antigens, detected by immunofluorescence; CF = antibodies in complement fixation test. NT = Antibodies in virus neutralizing test; n.d. = not done; d = days; m = months; y = years.

This view is further supported by the fact that significant rises in CMV-EA-antibody titres were not only apparent in the *de novo* infected immunosuppressed patients, but also in the cases that were already infected at time of transplantation. CMV-EA antibodies seem therefore not exclusively restricted to a primary CMV infection, but can also signal activation of a CMV infection. The maintenance of high anti-CMV-EA-antibody titres during immunosuppressive treatment indicates also that the hosts' capacity to produce virus-directed antibodies is still intact. Indeed even higher titres are recorded than after primary infection in non-immunosuppressed cases. This applies not only to antibodies detected in immunofluorescence (The *et al.*, 1974), but also for antibodies detected with complement-fixation and virus-neutralization tests (Andersen & Spencer, 1969).

In conclusion our findings suggest that the host-virus relationship is reflected by the serological antibody patterns against distinct CMV antigens. Latent infection, present in the majority of healthy adults, is accompanied by antibodies against CMV-LA only. Results of this study suggest that CMV-EA antibodies indicate a recent infection or a state of active viral proliferation.

## REFERENCES

- ANDERSEN, H.K. & SPENCER, E.S. (1969) Cytomegalovirus infection among renal allograft recipients. *Acta med. Scand.* 186, 7.
- ANDERSEN, H.K. (1971) Cytomegalovirus neutralization by plaque reduction. *Arch. ges. Virusforsch.* 35, 143.
- CRAIGHEAD, J.E., HANSHAW, J.B. & CARPENTER, C.B. (1967) Cytomegalovirus infection after renal allotransplantation. *J.A.M.A.* 201, 725.
- DUWALL, C.P., CASAZZA, A.R., GRIMLEY, P.M., CARBONE, P.P. & ROWE, W.P. (1966) Recovery of cytomegalovirus from adults with neoplastic disease. *Ann. int. Med.* 64, 531.
- KÄÄRIÄINEN, L., PALOHEIMO, J., KLEMOLA, E., MAKELA, T. & KOIVUNIEMI, A. (1966) Cytomegalovirus mononucleosis: Isolation of the virus and demonstration of subclinical infection after fresh blood transfusions in connection with open-heart surgery. *Ann. Med. exp. Fenn.* 44, 297.
- KRECH, U.H., JUNG, M. & JUNG, F. (1971) *Cytomegalovirus Infections in Man*. P. 36-39. S. Karger, Basel.
- SPENCER, E.S. (1974) Clinical aspects of cytomegalovirus infection in kidney-graft recipients. *Scand. J. infect. Dis.* 6, 315.
- STERN, H., ELEK, S.D., BOOTH, J.C. & FLECK, D.G. (1969) Microbial causes of mental retardation. The role of prenatal infections with cytomegalovirus, rubella virus and toxoplasma. *Lancet*, ii, 443.
- THE, T.H., KLEIN, G. & LANGENHUYSEN, M.M.A.C. (1974) Antibody reactions to virus-specific early antigens (EA) in patients with cytomegalovirus (CMV) infection. *Clin. exp. Immunol.* 16, 1.
- WELLER, T.H. & HANSHAW, J.B. (1962) Virological and clinical observations on cytomegalic inclusion disease. *New Engl. J. Med.* 266, 1233.
- WELLER, T.H. (1971) The cytomegaloviruses: ubiquitous agents with protean clinical manifestations. *New Engl. J. Med.* 285, 203 and 267.