CYTOMEGALOVIRUS ANTIBODY IN THE URINE OF RENAL TRANSPLANT PATIENTS

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CYTOMEGALOVIRUS (CMV) infection occurs in 32–96% of renal allograft recipients, the diagnosis usually being made by finding high serum antibody titres or viruria or both (Kanich and Craighead, 1966; Fiala *et al.*, 1975). Many of these infections are asymptomatic but can be associated with pneumonitis (Kanich and Craighead, 1966; Rifkind, Goodman and Hill, 1967; Fine *et al.*, 1972), hepatitis (Armstrong, Ely and Steger, 1971; Fine *et al.*, 1972), retinitis (Porter *et al.*, 1972), hyperpyrexia and transplant rejection syndrome (Lopez *et al.*, 1974).

Recent evidence that an infectious process involving the urinary tract can lead to local antibody production (Miller and North, 1971; Holmgren and Smith, 1975) prompted us to search for CMV antibody in the urine of renal transplant patients and to attempt to relate its presence to viruria.

MATERIALS AND METHODS

Patients. Sixteen patients who had received a kidney graft 4 months to 7 years previously were studied over a period of 9 months. The age range was 6–43 years, with a mean of 25, and there were 15 males and one female. They were all being treated with corticosteroids, 15–40 mg per day, and azathioprine, 50–150 mg per day.

Fifteen other patients, 23–47 years old, served as controls; three had chronic hepatitis, two were being investigated for repeated abortions, and 10 were attending an outpatients' clinic for a routine health-check examination.

Virus isolation. Urine from each patient was centrifuged at 3000 r.p.m. for 15 min. and the supernate, treated with penicillin and streptomycin to final concentrations of 100 units and 50 µg respectively per ml, was inoculated, in 0.2-ml amounts, into three tissue culture tubes of primary human-embryonic fibroblasts. The presence of CMV was recognised by the characteristic cytopathic effect and was confirmed by indirect immunofluorescence with human serum known to contain CMV antibody.

Testing for antibody in urine and serum. Urine samples were dialysed against 10 volumes of 0.1M phosphate-buffered saline (PBS), pH 7.2, for 36 h at 4°C. They were then concentrated 100-fold, by lyophilisation and reconstitution with PBS. The concentrated samples were again dialysed at 4°C against 10 volumes of PBS, with three to four changes of the PBS over a period of 24 h or until the sample became completely transparent. To avoid bacterial overgrowth during the processing, 0.01% of merthiolate was added initially to the urine samples. At least three samples, obtained at 15-day intervals, were examined from each patient. Serum was also collected from each patient, when the first urine sample was taken, and was stored at –20°C.

Concentrated urine and serum were titrated for CMV antibody by the microtitre complement-fixation (CF) test (Benyesh-Melnick, 1974), with commercially available antigen, and for antibody against Epstein-Barr virus (EBV) capsid antigen (EB-VCA) by indirect immunofluorescence (IF), with acetone-fixed lymphoblastoid cells of the carrier line P3HR-1 (Henle and Henle, 1966).

Urinary protein. Protein was estimated, in the 100-fold concentrated urine samples, by the biuret method (Hamburger *et al.*, 1966).

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RESULTS

The 15 control patients all had CMV antibody in their serum; the titres were 40 in six of them, 80 in four, 160 in three and 320 in two. None was excreting CMV nor could CMV antibody be detected in any concentrated urine sample, even by the CF test with undiluted antigen. The urinary protein was within physiological limits (Poortmans and Jeanloz, 1968), i.e., undetectable in the 100-fold concentrated urine of 13 patients and at the very low levels of 0.11 g and 0.14 g% in two patients.

Fifteen of the 16 kidney transplant patients possessed CMV antibody in their serum (table). Six were excreting CMV in the urine, and this was persistent in the five who were examined repeatedly at intervals of 1-2 months.

Eleven patients, including the six virus excretors, had CMV antibody in their urine, at titres of 1 to 64; similar results were obtained with all three urine specimens examined from each patient. Five of these patients (numbers 7-11 in the table), including only one of the virus excretors, also had EBV antibody in their urine, as well as high levels of urinary protein (1.24-8.36 g %). The other six (numbers 1-6) had no detectable EBV antibody in their urine, despite high titres in serum (64-256), and the urinary protein content was low (0.06-0.61 g % or less).

DISCUSSION

The kidney can synthesise immunoglobulins after a local antigenic stimulus (Miller and North, 1971; Holmgren and Smith, 1975). Viral antibodies have been detected in urine against BK virus, in patients receiving chemotherapy for malignancy (Reese *et al.*, 1975), and against papovavirus SV40, in experimentally infected monkeys (Ashkenazi and Melnick, 1962; Shah *et al.*, 1969). The present study describes local CMV antibody production in renal transplant patients excreting CMV.

TABLE

Tests for cytomegaloviruria, proteinuria and cytomegalovirus (CMV) and Epstein-Barr virus (EBV) antibodies in 16 renal transplant patients

Patient (number)	Presence or absence of CMV in urine	Antibody titre† against				Protein content of urine (g %)
		CMV		EBV		
		in serum	in urine‡	in serum	in urine‡	
1	+ (3/4)*	256	4	64	0	0.48
2	+ (4/4)	256	1	256	0	<0.01
3	+ (6/6)	256	4	128	0	0.06
4	+ (6/6)	256	8	128	0	0.61
5	+ (1/1)	128	2	64	0	0.24
6	— (0/2)	64	1	128	0	<0.01
7	+ (4/4)	64	32	256	128	8.36
8	— (0/3)	64	64	64	32	7.30
9	— (0/5)	256	64	64	32	3.70
10	— (0/4)	32	16	256	32	5.20
11	— (0/4)	256	8	256	8	1.24
12	— (0/3)	128	0	...	...	<0.01
13	— (0/3)	0	0	...	...	0.32
14	— (0/3)	64	0	...	...	<0.01
15	— (0/2)	16	0	...	...	0.21
16	— (0/2)	128	0	...	...	3.50

* Figures in parenthesis indicate the proportion of urine samples examined that yielded CMV.

† Complement-fixing antibody titre for CMV and immunofluorescent-antibody titre for EBV.

‡ The samples were concentrated 100-fold.

... = Not done.

Five patients (numbers 7-11) had raised levels of protein and both CMV antibody and EBV antibody in the urine. We believe that this indicates leakage of antibody from the blood. On the other hand, five of the six patients who were excreting CMV (numbers 1-5) had only CMV antibody in the urine, without significant proteinuria, although they had the same EBV antibody titres in their serum as patients 7-11; it should be noted that the IF test for detecting EBV antibody in urine is more sensitive than the CF test for CMV antibody. None of the remaining patients (numbers 12-16), nor any of the controls, had CMV antibody in their urine although their serum titres were comparable with those of the patients with urinary antibody, and some of them also had traces of protein in the urine.

Although the number of patients studied was small and the amounts of antibody detected in the urine were very low, the results were consistently obtained in repeated experiments and are compatible with local synthesis of antibody associated with growth of CMV in the kidney. However, much larger numbers of patients and controls must be examined, and future studies should include a greater clinico-pathological variety of patients excreting CMV. Careful evaluation of proteinuria will be required to confirm that growth of CMV in the kidney leads to local production of antibody, rather than to leakage of serum antibody resulting from kidney damage by the virus. Tests for antibody in urine may provide a further diagnostic approach to the study of active CMV infections of the kidney.

SUMMARY

Small amounts of cytomegalovirus (CMV) antibody were detected in the urine of renal transplant patients excreting the virus. The antibody was probably produced locally, as a result of active CMV infection of the urinary tract.

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