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ATTEMPTS TO ISOLATE BK VIRUS FROM CHILDREN AFFECTED BY VARIOUS DISEASES

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Summary. — Attempts to isolate BK virus (BKV) from specimens (urine, faeces, throat and nasal swabs and cerebrospinal fluid) obtained from 150 children affected by various diseases failed. Sera from the same children contained in 60.7 % of cases high titres of antibodies to BKV.

Key words: polyoma virus; childhood infection; antibodies

After the original isolation of BK virus (BKV) from the urine of a renal allograft recipient receiving immunosuppressive therapy (Gardner *et al.*, 1971), numerous reports confirmed strong association between human papovavirus infection and immunosuppression.

To define the route of transmission of BKV from infected to normal people, and to correlate the primary infection to a well-defined illness, we attempted to isolate the virus from specimens obtained from 150 children affected by various diseases; in addition, we have examined the sera of these children for antibodies to BKV.

Children, aged from 0 to 10 years, were all patients at the paediatric clinic. At the time of admission into the clinic, specimens of urine, faeces, throat and nasal swab, cerebrospinal fluid (CSF) and serum were taken from each child. All the specimens were immediately frozen and kept frozen until examined. For inoculation into Vero cell cultures, urine specimens were centrifuged and the sediments resuspended in Hanks' balanced salt solution (HBSS) containing 800 μg/ml penicillin plus streptomycin and 0.5 μg/ml amphotericin; faeces were suspended in HBSS plus antibiotics, centrifuged and the supernatant fluids used; throat and nasal swabs were washed in HBSS plus antibiotics; CSF was inoculated untreated. Vero cells were grown and maintained in Eagle's medium supplemented with 5 % and 1 % calf serum respectively, and 50 μg/ml gentamicin. Cells were cultivated in roller tubes and infected when they reached confluence. Four tubes were used for each specimen. The specimens were left to adsorb at 37 °C for 2 hr, then maintenance medium was added and the cells were further incubated for 14 to 30 days. Controls consisted of cells infected with BKV, and cells mock-infected with HBSS. BKV (Gardner strain) was generously provided by M. Portolani (Institute of Microbiology and Virology, University of Bologna, Italy). Since this BKV produced no cytopathic effect in Vero cell cultures, to detect the presence of virus in inoculated cultures, the tubes were frozen and thawed three times. The disrupted cells were then treated with receptor destroying enzyme (RDE) to improve virus release as follows: 4.5 ml of the cell homogenate, adjusted to pH 5.1 with 1 M HCl, were mixed with 0.5 ml of RDE, incubated at 37 °C overnight, adjusted to pH 7.0 with 1 M NaOH, heated at 56 °C for 1 hr and centrifuged at low speed to sediment cell debris. To detect BKV in the supernatant fluid, the haemagglutination (HA) test with human type 0 erythrocytes was

carried out according to Portolani *et al.* (1974). When HA was positive, haemagglutination inhibition (HI) tests with immune serum (Portolani *et al.*, 1974) and electron microscope examinations were performed. The HI was also used to detect the presence of antibodies to BKV in the sera.

BKV was not isolated from any child while high titres of antibodies were present in 60.7 % of the sera examined, with no correlation to any specific disease (Table 1).

Table 1. HI antibodies to BK virus in sera of children with different diseases

Disease	Proportion of sera with HI antibodies*	Range of titres**
Respiratory diseases	24/51 (47.1 %)	128—2048
Digestive diseases	21/29 (72.4 %)	512—1024
Haematological disorders	10/16 (62.5 %)	128—2048
Rheumatic disease	10/12 (83.3 %)	256—2048
Endocrine diseases	9/12 (75.0 %)	128—1024
Urinary disease	7/11 (63.6 %)	128—1024
Fever	5/5 (100 %)	128—4096
Neoplasia	1/4 (25.0 %)	2048
Disease of the central nervous system	1/4 (25.0 %)	1024
Distrophy	1/4 (25.0 %)	512
Liver diseases	2/2 (100 %)	128—1024
Total	91/150 (60.7 %)	

*Only titres $\geq$ 128 were considered specific.
**Only in sera considered positive.

Our failure to isolate BKV from 150 children may have been due to the fact that BKV multiplies very slowly in cultured cells and is not readily detectable. Our results on BKV epidemiology, however, confirmed the results obtained by other workers. More extensive investigations on the role of BKV in human disease are necessary.

References

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