PREVALENCE IN ITALY OF ANTIBODIES TO A NEW HUMAN PAPOVAVIRUS (BK VIRUS)

M. PORTOLANI, A. MARZOCCHI, G. BARBANTI-BRODANO AND M. LA PLACA

*Institute of Microbiology, University of Bologna, Via San Giacomo 12,
I-40126 Bologna, Italy*

BK VIRUS is a papova-like virus isolated by Gardner *et al.* (1971) from the urine of a patient on immunosuppressive therapy for kidney transplantation. Viruses with similar characters have been isolated from brain tissue from several cases of progressive multifocal leukoencephalopathy (PML) (Padgett *et al.*, 1971; Weiner *et al.*, 1972). BK virus is antigenically related to simian virus 40 (SV40) (Gardner *et al.*, 1971; Takemoto and Mullarkey, 1973), but is antigenically distinct from polyoma virus and human wart virus (Gardner *et al.*, 1971). It agglutinates human group-O erythrocytes and the haemagglutinin is firmly associated with the virion (Mäntyjärvi, Arstila and Meurman, 1972). It was shown recently to cause a malignant transformation of hamster cells (Major and Di Mayorca, 1973), thereby raising the question of a possible role in human oncogenesis.

Infection with BK virus in man is common in England (Gardner, 1973); haemagglutination-inhibiting (HI) and complement-fixing antibodies were found in all age groups. We investigated 453 healthy people in Italy by the HI test, and also by the fluorescent-antibody (FA) technique to detect antibodies to other structural components of the virion.

MATERIALS AND METHODS

Virus and cells. BK virus was kindly supplied by Dr S. D. Gardner and was grown in Vero cells. Growth and maintenance media were Eagle's minimum essential medium supplemented with 2.5% foetal bovine serum. One week after infection of the cultures the medium was changed and after a further 7–10 days' incubation, when cytopathic effects were clearly evident, cells and medium were frozen and thawed once, treated for 2 min. in a Branson ultrasonic disintegrator, and centrifuged at 800 g for 10 min. to sediment cellular debris. The supernatant fluid had a titre of 2048 to 40,768 haemagglutinating units (HAU) per ml, and was used as antigen in the haemagglutination (HA) and HI antibody tests. SV40 was grown in Vero cells in the same way as BK virus.

Sera. Serum specimens were obtained from healthy donors, ages 19–65 years, in the Blood Centre of the University Hospital, Bologna. Other sera were collected from healthy children and young people, aged 6 months–18 years, in Milan.

Haemagglutination. HA and HI antibody tests were performed in disposable plates by the microtitre method (Sever, 1962). Serial two-fold dilutions of BK virus were made in 0.05-ml amounts of phosphate-buffered saline (PBS), pH 7.2. Human type-O erythrocytes, from healthy donors, were washed three times and suspended to a concentration of 0.5% in PBS. One volume of PBS and two volumes of 0.5% erythrocytes were added to each virus dilution. Plates were incubated at +4°C and the HA titre was read 4 hours later, when control erythrocytes in PBS only had completely sedimented. The highest dilution of antigen giving complete haemagglutination was considered to contain 1 HAU.

Haemagglutination-inhibition. Sera were heated at 56°C for 30 min. and treated with NaIO₄ to remove non-specific inhibitors. Serial, two-fold dilutions of serum, from 1 in 4 to 1 in 8192, were made in 0.05 ml amounts of PBS. One volume of PBS containing 8 HAU of antigen was added to each serum dilution and the mixtures were kept at room temperature for 1 hour. After this time, two volumes of 0.5% erythrocytes were added, the plates were incubated at +4°C for 4 hours, and the HI-antibody titres were read. From the correlation

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found between HI and FA tests, an HI-antibody titre of 128 or greater was regarded as positive.

Preparation of antiserum to BK virus. Guinea pigs were given subcutaneous injections of the supernatant fluid from infected cell cultures. These were repeated four times at weekly intervals and animals were bled 5 days after the last injection. The immune serum had an HI antibody titre of 1280.

Immunofluorescence. Vero cells grown on glass coverslips were infected with BK virus or SV40. Four days later, the cells were fixed in acetone and stained by the indirect FA technique, with fluorescein-conjugated goat antiserum to human immunoglobulins (Hyland). Human test sera were examined at a 1 in 2 dilution only. Specificity controls included

TABLE I

The frequency of haemagglutination-inhibiting (HI) and fluorescent antibodies to BK virus and the distribution of HI-antibody titres in different age groups

Age group (years)	Proportion (and percentage) of sera with		HI-antibody tests	
	HI antibody*	fluorescent antibody	Range of titres†	Percentage of sera with titres of 512 or greater
0-2	22/46 (47.8)	18/46 (39.1)	128-8192	36.9
3-5	38/61 (62.2)	30/59 (50.8)	128-8192	42.6
6-10	57/75 (76.0)	52/72 (72.2)	128-4096	42.6
11-15	21/27 (77.7)	19/26 (73.0)	128-2048	37.0
16-25	34/41 (82.9)	15/19 (78.9)	128-2048	46.3
26-35	47/74 (63.5)	20/29 (68.9)	128-2048	33.7
36-45	39/51 (76.4)	20/25 (80.0)	128-1024	29.4
46-55	28/46 (60.8)	14/22 (63.6)	128-1024	26.0
56-65	19/32 (59.3)	9/13 (69.2)	128-1024	21.8
Total	305/453 (67.3)	197/311 (63.3)		

* Only titres of 128 or higher were regarded as specific.

† In sera regarded as positive.

BK-virus-infected cells stained with guinea-pig antiserum to BK virus and fluorescein-conjugated rabbit-antiserum to guinea-pig IgG (Hyland).

RESULTS

The distribution of HI and fluorescent antibodies to BK virus at various ages is illustrated in table I. A high frequency of antibodies was found in the Italian population and there was substantial agreement between the results of the two tests. Antibodies were already prevalent by the age of 2 years (47.8% by the HI and 39.1% by the FA test) and maximum frequency was reached by 16-25 years (82.9% by HI and 78.9% by FA). A small decrease occurred after the age of 45 years although the prevalence was still high. The highest serum titres (8192) were found in children under 5 years old; HI antibody titres of 512 or greater were found in all age groups, and most commonly between 16 and 25 years. A slight sex difference in the distribution of antibodies was observed in adults: antibodies were found in 79.2% (HI test) and 77.9% (FA test) of males and in 62.5% (HI test) and 56.5% (FA test) of females.

Table II illustrates the correlation obtained between the HI antibody titres and a positive FA test in sera that were examined by both methods. Almost all the sera with HI antibody

titres of 256 and greater were also positive by the FA test; 75% of sera with a titre of 128 were positive. On the other hand, the majority of the sera with HI antibody titres of 64 or less were negative by the FA test. It was for this reason that sera were accepted as positive for BK virus antibodies by the HI test only when the titre was 128 or higher. The specificity of the FA test for detecting BK-virus antibody in human serum was confirmed by the identical nuclear fluorescence obtained in BK-virus-infected cells stained by either human sera or specific guinea-pig antiserum.

Ten sera that were positive for BK-virus antibody by both the HI and FA tests, obtained from persons over 56 years of age who had not been vaccinated against poliomyelitis, were also tested on SV40-infected cells; all gave positive fluorescence.

TABLE II

Correlation between haemagglutination-inhibiting (HI) antibody titres and a positive fluorescent-antibody (FA) test in human sera

HI-antibody titres	Number (and percentage) of sera with HI antibody giving a positive FA test
8192	2/2 (100)
4096	10/10 (100)
2048	21/21 (100)
1024	37/37 (100)
512	48/51 (94)
256	32/35 (91)
128	18/24 (75)
64	12/29 (40)
32	6/31 (19)
16	2/22 (9)
8	2/12 (16)
<8	1/31 (3)

DISCUSSION

Our results confirm that antibodies against this human papova-like virus are common in man. The prevalence in Italy of BK-virus antibody is very similar to that found in England (Gardner, 1973), and to that of the closely related JC virus in the United States (Padgett and Walker, 1973).

The discrepancy between the HI and FA tests in some of our sera is most probably due to incomplete removal of non-specific inhibitors of HA. However, it is possible that the FA test is somewhat more sensitive because it also detects structural virus components that do not participate in the HA reaction.

BK virus is antigenically related to SV40, and SV40 antibodies have been found in persons given early batches of poliovaccine that were contaminated with SV40 (Gerber, 1967). However, our finding of such antibody in older persons who have no history of polio-vaccination suggests that these are actually due to the widespread prevalence of BK virus in the population (Shah *et al.*, 1971).

SUMMARY

Sera from 453 healthy persons of various ages were examined by haemagglutination-inhibition and by immunofluorescence for antibodies to BK virus, a new human papovavirus. Both tests showed that antibody was common in the population and that a majority of the infections probably occurred in early childhood.

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While this paper was in the press, a paper by Shah *et al.* (1973, *J. infect. Dis.*, **128**, 784), which reports results very similar to ours, came to our notice.

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