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Age-dependent prevalence of BK virus IgG and IgM antibodies measured by enzyme-linked immunosorbent assays (ELISA)

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SUMMARY

Enzyme immunoassays (ELISA) have been developed for the detection of BK virus IgG- and IgM-antibodies. Specific IgG is detected by an antigen-coated solid phase test; IgM by an antibody capture method.

These methods have been used to study the age-distribution of BK virus antibodies in Troms county in Northern Norway. The serum panels tested were: (*a*) 60 sera from paediatric patients aged 0–1 year; (*b*) 220 sera from healthy persons aged 1–82 years; (*c*) 74 sera from healthy blood donors; (*d*) 107 sera from healthy pregnant women.

The age-distribution of BKV-IgG antibodies showed that primary infections took place predominantly between the ages of 1 and 6 years, and that there were no sex differences, either in the age-specific prevalence or in the level of BKV-IgG.

We found no significant differences in the prevalence of BKV-IgM antibodies in healthy children and adults and pregnant women. BKV-IgM was detected in 26 of the 461 sera tested (5·6%).

INTRODUCTION

The human polyomavirus BK (BKV) was first isolated from the urine of a renal allograft patient (Gardner *et al.* 1971). This virus is potentially oncogenic and causes tumours when injected into hamsters (Mäntyjärvi, 1979). Seroepidemiological studies have shown that BKV has a world-wide distribution (Brown, Tsai & Gajdusek, 1975) and that the primary infection occurs predominantly in childhood with a peak incidence between the ages of 2–5 years (Dei *et al.* 1982; Gardner, 1973; Shah, Daniel & Warszawski, 1973). The virus may remain latent for a long time with occasional reactivation during pregnancy, immunosuppression and other pathological disturbances. Serological surveys have shown that most adults have been infected and that most children are born with passive antibodies which wane rapidly and are replaced with antibodies actively made in response to unrecognized infections (Dei *et al.* 1982). The primary infection is usually

symptomless, but an acute respiratory illness has been observed together with seroconversion to BKV (Goudsmit *et al.* 1982).

BKV-specific IgM antibodies have been demonstrated in pregnant women by Gibson *et al.* (1981), healthy adults and renal transplantation patients by Flower, Banatvala & Chrystie (1977), and in immunocompromised and healthy children by Rziha *et al.* (1978). The methods commonly used to demonstrate BKV-IgM are haemagglutination inhibition (HAI) and immunoelectronmicroscopy of sucrose density gradient fractionated sera (Gibson *et al.* 1981), or by immunofluorescence on whole sera (Rziha, Bornkamm & zur Hansen, 1978).

We have recently developed ELISA-tests to detect BKV-IgG and -IgM serum antibodies (Flaegstad & Traavik, 1985*a*,*b*). The aim of this investigation was to study the age-dependent prevalence of BKV IgG and IgM antibodies, and the relation between them.

MATERIALS AND METHODS

ELISA-tests for BKV-IgG and BKV-IgM

Details of the test for BKV-IgG (a solid phase ELISA) have been published already (Flaegstad & Traavik, 1985*a*). To examine whether the IgG results were influenced by concomitant IgM, a serum containing BKV-IgG (573 optical density (OD) units) and without BKV-IgM was mixed with an equal volume of a serum strongly positive for BKV-IgM (1084 OD units), but negative for BKV-IgG. Diluent was added to give a final dilution of 1 : 160 for both sera. The results showed that BKV-IgG was not influenced by concomitant BKV-IgM. The IgM test is an IgM antibody capture ELISA (Flaegstad & Traavik, 1985*b*). The test is not influenced by rheumatoid factor, and concomitant BKV-specific IgG does not interfere with the results.

Sera were tested in duplicate wells in a single dilution (1 : 160) for both tests. The levels of specific BKV antibodies are given in OD units relating the activity to two strongly positive standard sera, in which the level of IgG antibodies was set at 573 OD units and for IgM antibodies at 1084 OD units, respectively. The cut-off value, at which sera showing higher OD values were considered positive, was 60 OD units for IgG and 180 OD units for IgM.

Serum samples

Specimens were collected and stored at −20 °C; sera from four different groups were examined:

(*a*) 60 sera from children aged 0–1 year. The samples were taken from in- and out-patients suffering from a variety of illnesses at the Department of Paediatrics.

(*b*) 220 sera from healthy people aged 1–82 years. The sera were taken at health controls in Tromsø, and none of the persons had any acute or chronic illness at the time.

(*c*) 74 healthy blood donors aged 16–61 years visiting the blood bank.

(*d*) 107 healthy pregnant women aged 18–42 years. The sera came from Troms county and were routinely taken to examine for syphilis, rhesus-antibodies and rubella immune status.

Statistics

Differences between antibody prevalence in the groups were calculated by Fischer's exact test (two-sided) and differences in levels of OD units according to Wilcoxon's rank sum test.

RESULTS

BKV-IgG

Previously, a cut-off level of 40 OD units for BKV-IgG was used (Flaegstad & Traavik, 1985*a*), but from comparison with two HAI-tests, a level of 60 OD units would have been equally valid. When comparing the prevalence of BKV-IgG in the age-group 7–12 months, we found 41 % using a cut-off level of 40 OD units and 24 % using 60 OD units. From what is known about BK virus seroepidemiology (see below), the cut-off level was set at 60 OD units.

The age-distribution of BKV-IgG is shown in Table 1 and Fig. 1, which summarizes the results from serum panels a and b. The high percentage (75 %) of children aged 0–2 months having antibodies to BKV reflects passive transfer of IgG antibodies from their mothers. The lowest prevalence was found between 7–12 months (24 %). Thereafter a steady increase to 15 years of age was observed, when about 80 % had IgG antibodies to BKV. The distribution of strongly positive sera (OD units $\geqslant 300$) was similar, with the highest prevalence detected in the 5–12 years age group. Those over 60 years gave results similar to other adults. There was no sex difference in the prevalence ($P > 0{\cdot}5$) or the level (($P = 0{\cdot}45$) of BKV-IgG antibodies.

Among the blood donors aged 16–61 years, 84 % had IgG antibodies against BKV. In serum panels b and c we examined sera from 45 women aged 18–42 years. Of these, 80 % were positive for BKV-IgG compared to 87 % of the pregnant women ($P = 0{\cdot}40$). The geometric mean titer was 172 OD units compared to 221 in the latter group ($P = 0{\cdot}15$).

BKV-IgM

The age-distribution of BKV-IgM is shown in Table 2. None of the 60 children aged 0–1 years had IgM antibodies. Among 119 healthy persons aged 1–15 years, 11 % had BKV-IgM antibodies, while the proportion fell to 6 % in the 101 sera from adults aged 16–82 years. The difference is not significant ($P = 0{\cdot}28$), and neither is the sex difference observed ($P = 0{\cdot}13$). Of the 19 BKV-IgM positive sera, 17 also contained BKV-IgG, while 2 were negative. The 19 positive sera had a significantly higher level of BKV-IgG than the 201 BKV-IgM negative sera ($P = 0{\cdot}048$). However, if the 63 sera-negative for BKV-IgG were excluded, the 17 BKV-IgM containing sera did not significantly differ from the other sera in the level of BKV-IgG ($P = 0{\cdot}45$).

Three of the 107 pregnant women and 3 of the 45 women in the same age group had BKV-IgM ($P = 0{\cdot}48$). All the seven samples in serum panels c and d containing BKV-IgM also contained BKV-IgG. The levels of BKV-IgG in these sera were not significantly higher than for the BKV-IgM negative sera in panels c and d ($P = 0{\cdot}42$).

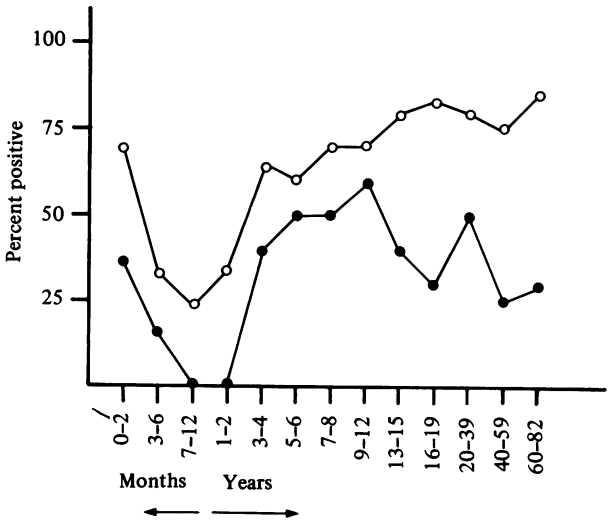

Fig. 1. Age-specific prevalence of BK virus IgG antibodies measured by ELISA. ○, Prevalence of positive sera (⩾ 60 OD units). ●, Prevalence of strongly positive sera (⩾ 300 OD units).

Table 1. *Prevalence and levels of BKV-IgG*

	Number	No. pos. IgG	Percent pos. IgG	OD units (Geom. mean)
Children 0–1 year	60	28	47	35
Children 1–15 years	119	76	64	77
Adults 16–82 years	101	81	80	126
Female 1–82 years	112	80	71	100
Male 1–82 years	108	77	71	94
Healthy blood donors	74	62	84	162
Pregnant women	107	93	87	221

Table 2. *Prevalence of BKV-IgM*

	Number	No. pos. IgM	Percent pos. IgM
Children 0–1 year	60	0	0
Children 1–15 years	119	13*	11
Adults 16–82 years	101	6*	6
Female 1–82 years	112	6	5
Male 1–82 years	108	13	12
Healthy blood donors	74	4	5
Pregnant women	107	3	3

* The age-distribution of patients with BKV-IgM was 6 in the age-group 1–4 years, 4 between 5–9 years, 3 between 10–14 years, 3 between 15–29 years, and the others 30, 70, and 82 years old.

DISCUSSION

Most of the previous seroepidemiological studies of BKV have been based on the HAI test. Several authors have found that low HAI titres may be due to incomplete removal of non-specific inhibitors rather than antibodies. Dei *et al.* (1982) argued for the use of an immuno-electrophoretic method to eliminate the influence of these factors. We have preferred to develop two separate enzyme immunoassays (ELISA), because these assays can determine very accurately levels of specific IgG and IgM antibodies for large numbers of sera.

The age-distribution of BKV-IgG is similar to the results previously reported by others (Dei *et al.* 1982; Gardner, 1973; Shah *et al.* 1973). As expected with passively transferred maternal immunity, the prevalence rates drop until an active humoral response to infection is superimposed (Fig. 1).

There have been conflicting reports on the prevalence of BKV-IgM antibodies in healthy individuals. Two studies, both employing sucrose density gradient fractionation followed by HAI, have reported BKV-IgM in 13/66 healthy adults (Flower *et al.* 1977) and 0/107 blood donors (Jung *et al.* 1975). Brown *et al.* (1984) used an IgM capture radioimmunoassay, and BKV-IgM was detected in 11/300 sera from blood donors (3·5 %), in 14/79 (18 %) and 24/114 (21 %) of sera from two groups of ill children. Compared with the blood donors in our panel c, there is no difference ($P > 0{\cdot}5$). Their second group were children aged 2–5 years whose sera were sent for estimation of antistreptolysin titres, and the children in the third group had various illnesses. Our results from healthy children aged 1–15 years do not differ from the results of the second group ($P > 0{\cdot}5$). The difference from the third group has a p-value of 0·052, and may be due to differences in the tests themselves; for example, from differences in setting the cut-off level or a higher prevalence of BKV-IgM in special patient groups.

We found no significant difference in the prevalence of BKV-IgM antibodies between children and adults. This is interesting because primary infection takes place in childhood, and these results could indicate reactivation, reinfection or longstanding IgM response. Longitudinal studies will be needed to investigate these possibilities.

Pregnant women did not have a significantly higher frequency of BKV-IgM than non-pregnant women but only one sample from each woman was examined at about 20 weeks gestation. In this group, the highest prevalence of BKV-IgM has been reported to be near term (Shah *et al.* 1980).

Of the 26 sera containing BKV-IgM, 24 sera also had BKV-IgG at levels similar to those in BKV-IgG positive/IgM negative sera. When judging the significance of BKV-IgM antibodies in a patient, the high prevalence in healthy individuals should be taken into consideration.

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REFERENCES

BROWN, D. W. G., GARDNER, S. D., GIBSON, P. E. & FIELD, A. M. (1984). BK virus specific IgM responses in cord sera, young children and healthy adults detected by RIA. *Archives of Virology* **82**, 149–160.

BROWN, P., TSAI, T. & GAJDUSEK, D. C. (1975). Seroepidemiology of human papovaviruses. Discovery of virgin populations and some unusual patterns of antibody prevalence among remote peoples of the world. *American Journal of Epidemiology* **102**, 331–340.

DEI, R., MARMO, F., CORTE, D., SAMPIETRO, M. G., FRANCESCHINI, E. & URBANO, P. (1982). Age-related changes in the prevalence of precipitating antibodies to BK virus in infants and children. *Journal of Medical Microbiology* **15**, 285–291.

FLOWER, A. J. E., BANATVALA, J. E. & CHRYSTIE, I. L. (1977). BK antibody and virus specific IgM responses in renal transplant recipients, patients with malignant disease, and healthy people. *British Medical Journal* **2**, 220–223.

FLAEGSTAD, T. & TRAAVIK, T. (1985*a*). Detection of BK virus antibodies measured by ELISA assay and two haemagglutination inhibition methods. A comparative study. *Journal of Medical Virology* **16**, 351–356.

FLAEGSTAD, T. & TRAAVIK, T. (1985*b*). Detection of BK virus IgM antibodies by two enzyme-linked immunosorbent assays (ELISA) and a hemagglutination inhibition method. *Journal of Medical Virology* **17**, 195–204.

GARDNER, S. D., FIELD, A. M., COLEMAN, D. V. & HULME, B. (1971). New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet i*, 1253–1257.

GARDNER, S. D. (1973). Prevalence in England of antibody to human polyomavirus (B.K.). *British Medical Journal* **1**, 77–78.

GIBSON, P. E., FIELD, A. M., GARDNER, S. D. & COLEMAN, D. V. (1981. Occurrence of IgM antibodies against BK and JC polyomaviruses during pregnancy. *Journal of Clinical Pathology* **34**, 674–679.

GOUDSMIT, J., WERTHEIM-VAN DILLEN, P., VAN STRIEN, A. & VAN DER NOORDAA, J. (1982). The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. *Journal of Medical Virology* **10**, 91–99.

JUNG, M., KRECH, U., PRICE, P. C. & PYNDIAH M. N. (1975). Evidence of chronic persistent infections with polyomavirus (BK type) in renal transplant recipients. *Archives of Virology* **47**, 39–46.

MÄNTYJÄRVI, R. A. (1979). New oncogenic human papova-viruses. *Medical Biology* **57**, 29–35.

RZIHA, H. J., BORNKAMM, G. W. & ZUR HAUSEN, H. (1978). BK virus: I. Sero-epidemiologic studies and serologic response to viral infection. *Medical Microbiology and Immunology* **165**, 73–81.

RZIHA, H. J., BELOHRADSKY, B. H., SCHNEIDER, U., SCHWENK, H. U., BORNKAMM, G. W. & ZUR HAUSEN, H. (1978). BK virus. II. Serologic studies in children with congenital disease and patients with malignant tumors and immunodeficiencies. *Medical Microbiology and Immunology* **165**, 83–92.

SHAH, K. V., DANIEL, R. W. & WARSZAWSKI, R. M. (1973). High prevalence of antibodies to BK virus, an SV40-related papovavirus, in residents of Maryland. *Journal of Infectious Diseases* **128**, 784–787.

SHAH, K., DANIEL, R., MADDEN, D. & STAGNO, S. (1980). Serological investigation of BK papovavirus infection in pregnant women and their offspring. *Infection and Immunity* **30**, 29–35.