AGE-RELATED CHANGES IN THE PREVALENCE OF PRECIPITATING ANTIBODIES TO BK VIRUS IN INFANTS AND CHILDREN

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SUMMARY. Nearly 1000 sera from children were tested by immunoelectro-osmophoresis against BK virus, and age-specific prevalence rates were estimated from birth until the age of 12 years. Declining rates during the first 12 months showed the waning of passive immunity, which at birth reflects the mother's immune status. The changes of prevalence suggested that the peak incidence of primary infections occurred at about 2 years, with an estimated peak annual rate of 24.6%.

INTRODUCTION

Immunocompromised hosts, notably transplant patients, are often overtly infected with the newly described human papovaviruses, which might be responsible for secondary disease (Padgett and Walker, 1976; Gardner, 1977). The best studied of these viruses, BK and JC, are considered to be the cause of ureteric stenosis and of progressive multifocal leuco-encephalopathy. There is serological evidence that these viruses are widespread in most human populations (Brown, Tsai and Gajdusek, 1975; Gardner, 1973; Padgett and Walker, 1973) and remain latent for long periods, with occasional reactivation during pregnancy (Borgatti *et al.*, 1979; Coleman *et al.*, 1980), and immunosuppression (Hogan, Borden and McBain, 1980) and other pathological disturbances (Takemoto *et al.*, 1974).

Despite the experimental oncogenicity of BK and JC viruses, evidence that they might be involved in human tumours (Fiori and Di Mayorca, 1976) has not been confirmed (Corallini *et al.*, 1976; Israel *et al.*, 1978; Shah *et al.*, 1978), and the extent of their pathogenicity is not yet defined (van der Noordaa and Wertheim-van Dillen, 1977; *Lancet*, 1978; Rziha, Bornkamm and Hausen, 1978a; Cheeseman *et al.*, 1980; Donini *et al.*, 1981). Particularly intriguing are the questions relating to these viruses as infectious agents: how do they infect, where do they multiply, how are they excreted, are they responsible for primary diseases?

Definite answers to such questions can be obtained only after many more observations on first infections which, however, are difficult to detect. Serological surveys indicate that adults are immune, and that most children are born with passive antibodies, which wane rapidly and are replaced by antibodies actively made in

response to unrecognised infections (Gardner, 1973; Mäntyjärvi *et al.*, 1973; Padgett and Walker, 1973; Shah, Daniel and Warszawski, 1973; Portolani *et al.*, 1974; Merletti, 1976; Panà *et al.*, 1976; Rziha *et al.*, 1978b).

Subclinical infections and any indefinite illness caused by BKV can be diagnosed only by laboratory tests, e.g., by virus isolation, or by detection of seroconversion. Epidemiologically it would be reasonable to concentrate study on the age groups showing the highest incidence of infection. We report here the results of a cross-sectional serological survey planned to obtain precise information on the age of maximum change in antibody prevalence and, by inference, on the age of peak incidence of infection.

MATERIALS AND METHODS

Sera. During September 1979–March 1980, about 50 consecutive serum samples for each age class, and a separate group of sera from immature babies, were separated from routine specimens of blood at the A. Meyer Children's Hospital, Florence; each patient's name, sex, age, and diagnosis were recorded. The criterion for defining immaturity was a birth weight of less than 2500 g. The sera were stored at -20°C ; when all had been collected they were tested in blocks, under code.

Immunoelectro-osmophoresis (IEOP). Technical details are to be published (Dei and Urbano, 1982). Briefly, 35 sera were placed in a row of anodal wells punched in a $100 \times 200 \times 1.25$ mm gel, and reacted against a strip (8×200 mm) of gel incorporating the antigen, at 20 mm, toward the cathode. The gel was made of agarose (Miles no. 95-201) 0.9%, polyethyleneglycol 6000 (Fluka) 3%, in tris-barbital-Nabarbital buffer, pH 8.8, μ 0.033 (HRB, Gelman); the same buffer was used in the electrophoresis tanks. Migration was effected at 50 V overnight. Plates were then washed, dried and stained. Reading of results was deferred until the end of the screening.

Individual results were read and graded on an ordinal five-point scale, from 0 to 4+, based on the appearance, intensity and position of the cathodal band, by comparison with the corresponding bands obtained with various dilutions of a reference antiserum.

Antigen. The Gardner strain of BKV was propagated on Vero cells. A single large pool of unconcentrated fluids from frozen and thawed degenerating cultures was prepared and stored frozen. The virus stock contained about 1000 haemagglutination units (HU) per ml; for IEOP one part of such antigen was mixed with 10 parts of molten gel and poured to fill the trough previously cut in the gel plate.

Haemagglutination inhibition (HI). Sera were heated at 56°C for 30 min; 5 μ l were mixed with 20 μ l of NaIO_4 (10mM) directly in microtitration wells. The reaction was stopped after 30 min at room temperature by the addition of 25 μ l of glycerol 0.63% (v/v). After 15 min, doubling dilutions were made in 25 μ l of PBS, starting with the reaction mixture, in which the serum was at a dilution of 10; 8 HU of virus were added in 25 μ l, and reacted for 60 min at room temperature. Plates were cooled at 4°C before the addition of cooled suspension human group-O erythrocytes 0.5% (v/v). Sedimentation patterns were read after overnight incubation at 4°C .

RESULTS

Precipitating antibodies to BKV were detected by IEOP in about half (486/984) of the sera examined; the prevalence was the same in males and females. The records revealed the expected variety of diagnoses, and we could find no connection between BKV antibody status and reason for admission to hospital or for blood sampling; in particular, there was no significant difference between prevalence rates of antibody in mature and immature babies (table I); the results were therefore grouped only by age group.

TABLE I

Prevalence of anti-BKV antibodies in mature and immature babies less than 1 month old

Babies	Number examined	Anti-BKV antibodies		
		present	absent	percentage prevalence
Immature	38	20	18	52.6*
Mature	66	31	35	47.0

* $\chi^2 = 0.309$; $p > 0.10$.

TABLE II

Grouping and number of sera tested in each age class, with distributions of graded IEOP reactivities

Age of children	Number of sera tested	Number of sera with IEOP reactivity of				
		0	1+	2+	3+	4+
0 months	104	53	13	15	16	7
1 month	45	25	6	7	6	1
2 months	51	37	5	6	3	0
3-5 months	48	34	9	1	4	0
6-8 months	46	35	9	1	1	0
9-11 months	45	36	5	3	0	1
12-17 months	62	53	4	1	2	2
18-23 months	42	32	8	2	0	0
24-29 months	45	32	3	0	8	2
30-35 months	46	24	5	4	7	6
3 years	44	24	4	2	10	4
4 years	43	16	1	5	13	8
5 years	49	17	2	6	16	8
6 years	45	13	3	9	18	2
7 years	45	10	9	4	20	2
8 years	44	12	7	4	19	2
9 years	43	15	6	2	17	3
10 years	49	10	9	5	23	2
11 years	40	8	7	9	15	1
≥ 12 years	48	12	5	12	16	3
All	984	498	120	98	214	54

IEOP = immunoelectro-osmophoresis.

Table II shows the grouping of sera, the number tested in each age group, and the distribution of their IEOP reactivities. Age groups were represented by subsamples of similar size, to yield prevalence estimates with a homogeneous sampling error; they were defined with a variable module, to cover the earlier ages at closer intervals.

The distributions of cumulated graded IEOP reactivities, that is, the percentage of sera showing at least 1+ (or 2+, 3+ . . .) IEOP reactivity, were determined for each age group and plotted on a graph (fig. 1); empirical frequencies are centred on the midpoint of their age interval and connected by a broken line; random fluctuations were attenuated by a simple mechanical perequation.

FIG. 1.—Age-specific prevalence rates of anti-BKV antibody reactivity by immunoelectro-osmophoresis (IEOP). ● = Strong, 4+, IEOP reactivity; ○, △ and □ = 3+, 2+ and 1+ reactivity, respectively.

Table III summarises the seroepidemiological features, as estimated in the population sampled. The prevalence of antibodies at birth was extrapolated from the empirical prevalences in the first age intervals, and may be compared with an independent estimate of 87.5%, obtained on a group of 24 cord-blood sera. These were examined alongside their mother's serum, and showed complete correspondence.

As expected of passive immunity, prevalence rates of antibody reactivity decreased regularly in the first 6 months, at which age more than 25% of children still had at least some reactivity, fewer had intermediate reactivity and none had strong reactivity. After the age of 6 months, the trend of prevalence reflects the continuing decline of passive immunity and the superimposed effect of active humoral response to infection. The latter is best represented in fig. 1 by the line showing the prevalence of strong, 4+, IEOP reactivity, which is indicative of recent infection.

The lowest antibody prevalence, 18.8%, was reached by 18 months; the following rise was steepest between 21 and 27 months, a semester accounting for an increase of

TABLE III
Seroepidemiological characteristics of BK virus infection

Prevalence of antibodies at birth: c. 75%.
Minimum prevalence: 18.8%.
Age of minimum prevalence: 15 months.
Period of maximum variation of antibody prevalence: from 21 to 27 months.
Inferred peak incidence: 24.6% per annum.
Inferred age of peak incidence: 2 years.
Modal age of strong antibody reactivity: 4 years.

12.3% in the prevalence of children with antibodies to BKV, during which children face the highest annual incidence of first infections, about 25%.

For older children, the upper line of fig. 1 best shows the prevalence of those who have had past experience of infection with the virus—about half of them by 4 years, about three quarters after 8 years. The other lines reflect the distributions of the graded IEOP reactivities and their changes with age. The prevalence line of strong, 4+, antibody reactivity is of epidemiological interest. It peaks at 4 years, that is, at this age children tend to have higher antibody levels; then it gradually declines, without reaching zero. Perhaps older people with high antibody levels are the ones who have experienced a recent reactivation.

DISCUSSION

Most previous surveys for the seroepidemiology of BKV were based on the reaction of haemagglutination inhibition (HI). Several authors (Portolani *et al.*, 1974; De Stasio *et al.*, 1980) and ourselves have found that low HI titres may be due to incomplete removal of nonspecific inhibitors rather than to antibodies, and prevalence estimates of BKV antibodies have been derived from rather arbitrarily chosen cut-off values. Published results of surveys made on various populations may not be properly compared because of lack of standardisation: modal HI titres are quite different from one study to another, even when they are based on large samples of comparable populations. To obviate these defects of the HI reaction, we set up the immunoelectro-osmophoretic method for the detection of BKV antibodies and have tested its validity by several criteria, including a comparison with HI on about 500 sera from the present series (Dei and Urbano, 1982).

The HI results were handled as detailed above, and are shown in fig. 2. They are directly comparable with those in fig. 1, based on IEOP. Here again the prevalence

FIG. 2.—Age-specific prevalence rates based on haemagglutination-inhibition (HI) titration. ● = HI titre of ≥ 640 ; ○, △ and □ = titre of ≥ 160 , ≥ 40 and ≥ 10 respectively (from Dei and Urbano, 1982).

lines at the various HI titres show a very steep decline during the first 3 months of life; from then on, the waning of passive immunity is masked by the active humoral responses of the babies. Very high HI titres are absent until 23 months; however, substantial titres (40 and 160) are present in 10–20% of sera by 12 months, thus showing that babies start to be infected at even earlier ages. A similar conclusion may be drawn by considering the upper prevalence line in fig. 2, which, however, might be biased by a background prevalence of nonspecific HI inhibitors. Inferences about the peak incidence of infection agree with those presented above.

We believe that the IEOP results are more reliable than HI; they suffer from being expressed on an arbitrary ordinal scale, which does not allow direct comparison with HI titres, but this is due mainly to the undetermined meaning of the latter; sera scoring 1+ most probably contain low concentrations of specific antibodies because the precipitates they develop with BKV antigen are exactly like those developed by very dilute reference hyperimmune sera. When the results of the whole series are considered, IEOP gives a more coherent picture of the age-related changes of antibody prevalence. The results indicate that a few children already have, actively, made precipitating antibodies to BKV by the age of 6 months.

The results of previous surveys were carefully considered when we planned the present one to obtain more precise estimates of the main sero-epidemiological features of BKV infections. We fully confirm that these infections are very common and are acquired early in childhood, soon after the waning of passive immunity; our more detailed findings relate to the particular population we have sampled, and they are, at best, indicative of the epidemiology of BKV infections in the target population of Florentine children. It will be interesting to compare our results with those from similarly detailed studies on populations with different epidemiological settings.

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