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Serological Investigation of BK Papovavirus Infection in Pregnant Women and Their Offspring

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Paired sera from 150 pregnant women and 387 umbilical cord sera were tested for BK virus (BKV) antibodies. The hemagglutination inhibition, neutralization, and indirect immunofluorescence tests were employed for the detection of antibodies. Treatment of serum with anti-γFc and tests of immunoglobulin M (IgM) fractions for antibodies were utilized as required to detect and validate the presence of virus-specific IgM. The BKV antibody prevalence in the sera collected at the time of the first prenatal visit was 75% by hemagglutination inhibition and 91% by neutralization tests. A total of 95% of the women had antibodies by at least one of the three serological tests. Five of 100 women with normal pregnancies exhibited BKV activity during pregnancy as evidenced by a greater than fourfold rise in BKV hemagglutination inhibition antibody titers and acquisition of BKV-specific IgM. The antibody rise occurred in the younger women and appeared to be a result of reactivation of the virus rather than of primary infection. Two instances of possible recent BKV infections were identified. BKV-specific IgM was not detected in any of the 387 umbilical cord sera which included three specimens from infants born to mothers with definite or probable BKV activity during pregnancy and 50 specimens with IgM levels of >20 mg/100 ml. The results indicate that few women in the child-bearing age are nonimmune to BKV and that, although reactivation of infection occurs in pregnancy, congenital transmission of the virus either does not occur or is rare.

Antibody surveys in different populations have shown that infection with the human papovavirus BK virus (BKV) is widespread and that most of the infections occur in early childhood (6, 14, 20). No illness is associated with the primary infection. Beginning with the initial report of recovery of BKV from the urine of a renal transplant recipient (8), almost all isolations of BKV have been made from individuals who were immunologically impaired, for example, renal and bone marrow transplant recipients, cancerous patients on immunosuppressive chemotherapy, and individuals with immune deficiency diseases (4, 7, 13). In 1975, Taguchi et al. (23) reported the results of a serological study in Japan which indicated that BKV infection was relatively common in pregnancy and that the virus was transmitted congenitally. They found BKV-specific antibodies of the immunoglobulin M (IgM) class in the umbilical cord sera of three of the six mothers who showed antibody rise to BKV during pregnancy. In 1978, Rziha et al. (17) reported that 9.1% of the umbilical cord sera collected in southern Germany had BKV-specific IgM antibodies and suggested that transplacental transmission of BKV was a frequent occurrence. However, Borgatti et al. (3) failed to detect BKV-specific IgM in umbilical cord sera. Most recently, Coleman et al. (5) demonstrated BKV excretion in the urine of pregnant women but did not find any evidence of transplacental transmission of the virus.

We describe here the results of an antibody survey of maternal and umbilical cord sera. A very small proportion of the women in early pregnancy was without demonstrable antibodies to BKV. A rise in BKV antibody titers occurred in some women during pregnancy. This rise appeared to be a result of virus reactivation rather than of primary infection. Reactivation was more likely at younger ages. An examination of the umbilical cord sera for BKV-specific IgM revealed no evidence for congenital transmission of the virus.

MATERIALS AND METHODS

Sera. Three groups of sera were tested.

(i) Normal pregnancies. A total of 100 paired specimens from women with normal pregnancies were examined to determine the antibody prevalence in the first specimens and for changes in antibody titer during pregnancy. As a rule, the first serum was taken at the

time of the first prenatal visit, and the second was taken at the time of childbirth. Umbilical cord sera from infants born to 37 of these women were available and were also tested. These sera were a part of the collections made during 1959 to 1965 for the nationwide Collaborative Perinatal Project (19).

(ii) Infants born with high IgM levels and their mothers. We examined 50 umbilical cord sera with IgM levels of >20 mg/100 ml. Infants born with such high levels of IgM are thought to represent a population in which congenital transmission is more likely to have occurred (1, 18). Paired specimens from mothers of these infants were also tested. These sera were also from the collections of the Collaborative Perinatal Project.

(iii) Normal infants. A total of 300 umbilical cord sera obtained from infants born at the University Hospital, Birmingham, Alabama, were tested. These were collected in the early 1970s during studies of congenital infections (22). The mothers of these infants comprised a young, predominantly black, low-income group from metropolitan Birmingham. Maternal sera for this group were not available.

Tests for BKV antibodies. (i) HAI tests. The sera were extracted with acetone, adsorbed with human O erythrocytes and titrated for hemagglutination inhibition (HAI) antibodies with 4 to 8 U of BKV hemagglutinin (20). All sera from an individual were examined in the same test. Serum fractions obtained after sucrose density gradient centrifugation were tested without any treatment.

(ii) Neutralization tests. The neutralization test is more sensitive than the HAI test for the detection of BKV antibodies (20) and was performed on first serum specimens of women with normal pregnancies. A single 1:5 dilution of serum was tested with 30 to 100 50% tissue culture infectious doses of BKV. Each serum-virus mixture was inoculated into two tubes of W138 fibroblasts, and the inoculated cultures were observed for BKV-specific cytopathic effect.

(iii) IF tests. Indirect immunofluorescence (IF) tests, using 1:5 dilutions of sera and BKV-infected W138 cells (20), were performed for the detection of BKV antibodies of the IgG and IgM classes. The intensity of the staining was graded on a scale of 1 to 4.

For IgG antibodies, after a 30-min incubation with serum, the infected cells were reacted with fluorescein-conjugated goat antihuman IgG (γ chain specific) (Meloy Laboratories, Springfield, Va.).

For IgM antibodies, after a 3-h incubation with serum, the infected cells were reacted with fluorescein-conjugated rabbit antihuman IgM (μ chain specific) (Dako Brand, Accurate Chemical and Scientific Corp., Hicksville, N.Y.). This conjugate was shown to be specifically reactive with IgM as follows. Six sera from renal transplant patients with cytological or virological evidence of BKV infection (M. D. Traystman et al., Acta Cytol., in press) were fractionated by sucrose density gradient centrifugation (see below). The IgG fractions of these sera had high titers of BKV HAI antibodies, but they did not stain BKV antigen in indirect IF tests when tested with the antihuman IgM. IgM fractions of two of the six sera had BKV HAI antibodies. These fractions stained BKV antigen in IF tests with the antihuman IgM.

Validation of tests for virus-specific IgM. The interpretation of IF tests for specific IgM is subject to two kinds of errors. Rheumatoid factors (anti-IgG antibodies of the IgM class), which are present in some sera, although they are nonspecific for the antigen, bind to antigen-specific IgG (if it is present in the serum) and so secondarily stain the antigen and give a false-positive reaction (15, 21). On the other hand, a high concentration of virus-specific IgG antibodies may interfere with the demonstration of virus-specific IgM antibodies in the same serum by competing for antigenic sites and thereby give a false-negative reading (2). It has been shown that separation of IgG and IgM fractions by sucrose density gradient centrifugation (16) or removal of IgG by adsorption with anti-γFc (9, 10) eliminates both of these problems. These methods were applied on selected sera.

(i) Fractionation of IgG and IgM. The serum was diluted 1:5 in phosphate-buffered saline (PBS) and spun at 2,500 $\times$ *g* for 30 min. A 0.2-ml sample of the supernatant was layered onto a 10 to 30% (wt/wt) linear sucrose gradient prepared in 0.01 M tris(hydroxymethyl)aminomethane-buffered saline (pH 7.4) with cellulose nitrate tubes, and centrifuged for 5.5 h at 50,000 rpm in a Beckman SW50.1 rotor. Fractions of about 0.3 ml each were recovered from the bottom of the tube and tested for BKV antibodies in HAI tests. The adequacy of the separation procedure was monitored by tests of fractions of one serum in each centrifugation run for the presence of IgG and IgM in radial immunodiffusion plates (Hyland Laboratories, Deerfield, Ill.).

(ii) Removal of IgG by adsorption with anti-γFc. Commercially prepared goat anti-γFc serum (Cappel Laboratories, Cochranville, Pa.) was rehydrated to 2$\times$ concentration. A 75-μl amount of the rehydrated reagent was added to 50 μl of a 1:2 dilution of serum in PBS, incubated for 1 h at 37°C and overnight at 4°C, and then centrifuged at 1,500 $\times$ *g* for 0.5 h. The supernatant was used for IF tests. This treatment reduced the IgG concentration by >95% without altering the concentration of IgM, as judged by radial immunodiffusion tests in commercially prepared immunodiffusion plates.

RESULTS

BKV antibody prevalence in sera of women in early pregnancy. BKV HAI antibodies were demonstrable in 75 of the 100 first bleedings from women with normal pregnancy (Table 1). The antibody titers in the positive sera ranged from 1:20 to 1:1,280 with a median value of 1:80. To detect low levels of antibodies which may be undetectable in HAI tests and to confirm the positive results of HAI tests, we also tested these sera for neutralizing antibodies (Table 1). Neutralizing antibodies were demonstrable in all but one of the 75 sera which had HAI antibodies; the single exception was a serum with an HAI antibody titer of 1:20. In addition, 17 (68%) of 25 sera with no demonstrable HAI antibodies were protective in neutralization tests. In IF tests, four of the nine sera without

detectable neutralizing antibodies stained the viral antigen. There were only five sera of women in early pregnancy which were negative for BKV antibodies by all three tests.

TABLE 1. *BKV antibody (Ab) prevalence in women in early pregnancy by HAI and neutralization tests*

No. of sera	HAI Ab titer[a]	No. of sera with neutralizing Ab[b]	
		Present	Absent
25	Negative	17	8
15	20	14	1
19	40	19	0
15	80	15	0
15	160	15	0
8	320	8	0
2	640	2	0
1	1,280	1	0

[a] Antibody prevalence, 75%.
[b] Antibody prevalence, 91%.

BKV activity during pregnancy. All maternal sera were titrated for HAI antibodies to BKV and were screened for BKV-specific IgM in indirect IF tests. Definite evidence of BKV activity during pregnancy, as judged by a fourfold or greater rise in HAI antibody titers, was demonstrated in five mothers (Table 2); all of them were from the group with normal pregnancies. The first serum in these cases was obtained at between 6 and 26 weeks of gestation, and the second serum was obtained at the time of delivery. All of the first sera had neutralizing antibodies, but two were without demonstrable HAI antibodies. BKV-specific IgG was present in both specimens of all five pairs by IF tests. On the other hand, in IF tests for BKV-specific IgM, these antibodies were detected in none of the first specimens but in all of the second specimens of the five pairs. In three of the five second specimens, the IgM was demonstrable only after removal of the IgG by anti-γFc treatment. In

TABLE 2. *Antibody response of women with definite or probable BKV activity during pregnancy and antibodies in three corresponding umbilical cord sera*

No.[a]	Age (yr)	Serum	Time of collection (source)	BKV antibody response					
				Neutralization	HAI		IF		
					Whole serum	IgM fraction[b]	IgG	IgM	
								Untreated serum	Treated with anti-γFc
Antibody rise during pregnancy									
1	20	1-1	6 Wk	Positive	320	N[c]	3+	N	N
		1-2	Term		1,280	40	4+	N	3+
			(Fetus-umbilical cord)		320	N	3+	N	N
2	18	2-1	11 Wk	Positive	40	N	1+	N	N
		2-2	Term		160	N	3+	1+	1+
3	20	3-1	24 Wk	Positive	N	N	1+	N	N
		3-2	Term		320	N	4+	N	0-1+
4	20	4-1	26 Wk	Positive	N	N	1+	N	N
		4-2	Term		320	40	4+	2+	2-3+
5	24	5-1	20 Wk	Positive	160	N	3+	N	N
		5-2	Term		640	N	4+	N	2+
Possible recent infection									
6	15	6-1	29 Wk	Positive	1,280	80	4+	0-1+	3+
		6-2	Term		640	160	4+	0-1+	3-4+
			(Fetus-umbilical cord)		320	N	4+	N	N
7	22	7-1	35 Wk	Positive	1,280	20	4+	0-1+	2+
		7-2	Term		1,280	40	4+	N	1+
			(Fetus-umbilical cord)		1,280	N	4+	N	N

[a] Numbers 1-6 were from the group of women with normal pregnancy, and number 7 was from the group with infants born with IgM level of >20 mg/100 ml.
[b] Most often, more than one IgM fraction had HAI antibodies; the highest titers are listed in this column.
[c] N, None.

IgM fractions of these pairs obtained after sucrose density gradient centrifugation, BKV HAI antibodies were detected in none of the first specimens but were found in two of the five second specimens, each with a titer of 1:40.

We found two additional instances of possible BKV activity during pregnancy, one in the normal pregnancy group and the other in the group with infants born with >20 mg/100 ml (Table 2). In both of these mothers, the first serum collected late in pregnancy, as well as the second serum collected at the time of delivery, contained high titers of BKV HAI antibodies and BKV-specific IgM. The demonstration of BKV-specific IgM in IF test was facilitated by removal of IgG by treatment with anti-γFc. Nine additional maternal sera showed some degree of IgM reactivity in IF tests of untreated sera, but the reactivity was abolished or greatly diminished after treatment with anti-γFc, and the IgM fractions of the sera were negative for BKV HAI antibodies.

BKV activation appeared to occur more frequently in younger mothers (Table 3). Among the 100 mothers with normal pregnancies, BKV activity was found in 6 of 55 women who were less than 26 years old and in none of 45 older women. Four of the six mothers with BKV activity were undergoing their first pregnancy.

Search for BKV-specific IgM in umbilical cord sera. BKV-specific IgM could not be unequivocally demonstrated in a single specimen in an examination of 387 umbilical cord sera.

Three of the cord sera corresponded to mothers 1, 6, and 7 in Table 2. None of these sera, either untreated or after treatment with anti-γFc, stained BKV antigen in IF test for IgM (Table 2). They had large amounts of BKV HAI antibodies in their IgG fractions but none in the IgM fractions. These data on two maternal-fetal pairs are shown in Table 4. The lack of BKV IgM antibodies in cord sera contrasted with their presence in the maternal sera at delivery.

All of the cord sera were titrated for BKV HAI antibodies and screened for IgM reactivity by IF tests with untreated sera (Table 5). BKV HAI antibodies were detected in 74% of the sera and were present in titers of 1:160 or above in 21% of the sera (80 specimens); six showed some IgM reactivity. The HAI antibody titers of these six sera ranged from negative to 1:1,280, and four had titers of 1:160 or above (Table 5, footnotes). Five of the six IgM-reactive sera were in the group of cord sera with IgM levels of >20 mg/100 ml. In carefully monitored and repeated tests, BKV-specific IgM antibodies could not be demonstrated in any of the six sera. Treatment with anti-γFc abolished the IgM reactivity in IF test, and BKV HAI antibodies could not be demonstrated in the IgM fractions (Table 6). Similarly, tests of 16 umbilical cord sera with HAI antibody titers of 1:640 or above revealed none which had HAI antibodies in their IgM fractions (Table 6). These 16 sera, as well as 57 sera with HAI antibody titers of 1:160 or 1:320, exhibited no IgM reactivity in IF tests after removal of the IgG by treatment with anti-γFc (Table 6).

TABLE 3. *Distribution of women showing BKV activity during normal pregnancy by age and number of previous pregnancies*

Age (yr)	No. of women with BKV activity					
	0[a]	1	2	3	4	≥5
15–18	2/6[b]	0/2				
19–22	1/10	0/8	1/3	1/1	0/1	
23–26	1/6	0/8	0/4	0/2	0/3	0/1
27–30	0/1		0/5	0/3	0/1	0/3
31–34			0/7	0/2	0/1	0/7
≥35			0/2	0/2	0/3	0/8

[a] Number of previous pregnancies.

[b] The numerator is the number of women showing BKV activity; the denominator is the number of women in that age and pregnancy category.

DISCUSSION

This investigation was stimulated by the report of congenital transmission of BKV by Taguchi et al. (23) and was aimed at estimating the proportion of pregnant women who are nonimmune to BKV, the frequency of BKV activity during pregnancy, and the frequency of congenital transmission. The antibody studies of the first serum specimens of the 100 women with normal pregnancies showed that there were few individuals in this group who were truly nonimmune and at risk of primary infection with BKV. Although the HAI antibody prevalence in this series was only 75%, the more sensitive neutralization test detected antibodies in 91%, and only five sera were without demonstrable antibodies when three different tests were employed. These data are in accord with the previous observation that BKV infection is acquired during childhood (6, 14, 20). In a U.S. population, the BKV HAI antibody prevalence rate reached 50% by the age of 3 to 4 years, rose to nearly 100% by the age of 10 to 13 years, and then dropped to 67% in the older age groups. The HAI antibody titers of positive sera declined with increase in age (20). It is therefore difficult to be certain whether the five women who remained seronegative through pregnancy represented individuals who were infected in the past and had declining antibody levels, or individuals who were susceptible to primary infection.

TABLE 4. *BKV HAI antibody titers in serum fractions of maternal and umbilical cord sera*[a]

Serum	BKV HAI antibody titer in serum fraction:[b]																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1-1	—	—	—	—	—	—	—	—	20	40	80	20	—	—	—	—	—	—
1-2	—	—	20	40	20	—	—	—	20	20	320	160	20	—	—	—	—	40
Cord	—	—	—	—	—	—	—	—	40	40	320	320	160	40	—	—	—	80
6-1	—	20	40	80	80	—	—	—	—	40	80	640	1,280	320	40	20	—	40
6-2	—	80	160	40	20	—	—	—	40	80	320	1,280	1,280	320	40	40	—	40
Cord	—	—	—	—	—	—	—	—	20	40	640	1,280	640	80	40	20	—	40

[a] The immunoglobulin class was determined by radial immunodiffusion assay of serum fractions. The sera are from cases 1 and 6 of Table 2.

[b] Immunoglobulin classes for serum fractions in columns 2–4 and 9–14 are IgM and IgG, respectively.

[c] —, None.

TABLE 5. *Umbilical cord sera: HAI antibody (Ab) titers and reactivity in preliminary IF test for IgM*

Source[a]	No. of sera	No. of sera with IgM reactivity[b]	Distribution of sera by HAI Ab titer		
			Negative	20–80	160–2,560
Normal pregnancy (CPP)	37	0	17	17	3
With IgM > 20 mg/100 ml (CPP)	50	5[c]	24	17	9
Normal pregnancy (Birmingham)	300	1[d]	60	172	68

[a] CPP, Collaborative Perinatal Project.

[b] In tests of untreated sera.

[c] The HAI antibody titers of these sera were: Negative, 1:40, 1:160, 1:640, and 1:1,280.

[d] The HAI antibody titer of this serum was 1:160.

TABLE 6. *Umbilical cord sera: results of tests for BKV-specific IgM*

Category	no. positive/no. tested	
	IgM reactivity after treatment with anti-γFc	HAI antibody in IgM fractions
Mothers with definite or probable virus activity in pregnancy	0/3	0.3
IgM reactivity in untreated serum	0/6	0/6
HAI antibody titer of 1:640 or greater	0/16	0/16
HAI antibody titers of 1:160 and 1:320	0/57	NT[a]

[a] NT, Not tested.

Five of the 100 women with normal pregnancy whose paired sera were examined exhibited definite evidence of BKV activity during pregnancy. In these cases, there was a significant rise in HAI antibody titer and acquisition of BKV-specific IgM during pregnancy. In a sixth case, the serological data suggested a recent infection. The BKV-specific IgM in these sera was not present in large amounts and was demonstrated readily only when the competing IgG antibodies were removed. The first specimen of all these cases had detectable BKV antibodies, suggesting that the antibody rise was a result of reactivation of virus rather than of primary infection. The true frequency of BKV reactivation during pregnancy is probably greater than the 5 to 6% found here. The time interval between the collection of the two specimens did not cover the period of early pregnancy, and a transient serological rise could have gone undetected because the two sera were collected several months apart. Although it is likely that the virus reactivation is a result of pregnancy, serological data from comparable nonpregnant females are not available. Cytological evidence of papovavirus infection is found in pregnant females but not in nonpregnant females (5).

The evidence for congenital transmission was sought by efforts to demonstrate BKV-specific IgM in umbilical cord sera. All of 387 cord sera were tested for HAI antibodies and were screened, untreated, for IgM reactivity in IF tests. A total of 305 sera which had HAI antibody titers of 1:80 or lower and were negative for IgM in the screening test were not studied further. In the evaluation of the remaining sera for BKV-specific IgM, two criteria were employed: tests of IgM fractions for BKV HAI antibodies and IgM reactivity in IF tests of sera from which IgG was removed by treatment with anti-γFc. The validity of these criteria is well established by tests for IgM antibodies to rubella virus (9, 16) and was evident in tests of maternal

sera for anti-BKV IgM (Table 2). Neither of the two criteria was met by the following groups of sera: (i) three sera from infants born to mothers for whom there was definite or probable evidence of virus activity during pregnancy: (ii) six sera which showed some IgM reactivity in the screening IF tests for BKV IgM; and (iii) 16 additional sera which had BKV HAI antibody titers of 1:640 or greater. The 57 remaining sera which had HAI antibody titers of 1:160 and 1:320 were negative for IgM, both in the screening IF test and in IF tests after treatment with anti-γFc.

Our data are at variance with the conclusions of two previous investigations, based on the demonstration of BKV-specific IgM umbilical cord sera, that congenital transmission of BKV occurs commonly (17, 23). The reasons for this discrepancy are not clear. On the other hand, our findings are similar to those of Borgatti et al. (3), who failed to detect BKV-specific IgM in umbilical cord sera in an Italian population. Coleman et al. (5) studied over 1,000 pregnant women prospectively and identified 40 individuals as excreting a papovavirus during pregnancy on the basis of urinary cytology. These investigators failed to detect BKV-specific IgM in the umbilical cord sera of infants born to these 40 mothers or of infants born to 270 cytologically negative mothers.

The studies of the pathogenesis of murine papovaviruses point to the importance of serum antibodies in preventing the dissemination of infection. McCance and Mims have found that in female mice infected at birth with polyoma virus, pregnancy reactivates the infection in the kidney but that the virus does not spread to other organs or to the fetus (12). In contrast, the virus can be transmitted transplacentally if pregnant females are infected for the first time during gestation (11). K virus-induced pulmonary pathology and mortality in infant mice are prevented by the administration of antibodies and of primed B cells, presumably as a result of neutralization of free virus in the serum (F. Shirazian, Ph.D. thesis, Johns Hopkins University). Taken together, the results from human and animal studies suggest that in previously infected individuals, papovaviruses may be reactivated in pregnancy but that the infection is unlikely to be transmitted to the fetus. In this respect, the papovaviruses differ from cytomegalovirus which is readily transmitted congenitally in immune mothers (22). Primary BKV infection during pregnancy may pose a greater risk of congenital transmission, but this seems to occur rarely as described in this report, as well as in other populations studied (3, 5).

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