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Serological Investigation of the Possibility of Congenital Transmission of Papovavirus JC

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In an examination of paired sera from pregnant women, 9 of 57 women who had JC virus antibodies in their first specimens exhibited virus reactivation, as judged by a fourfold or greater rise in JC virus hemagglutination-inhibiting antibodies; 43 women remained seronegative through pregnancy. JC virus-specific immunoglobulin M was not demonstrated in umbilical cord sera of six infants born to mothers showing reactivation or in 300 additional umbilical cord sera from normal pregnancies.

In 1975, Taguchi et al. (14) reported that infection with BK virus (BKV), a human papovavirus related to JC virus (JCV), is frequent in pregnant women and that the virus is transmitted congenitally. The evidence for congenital transmission of BKV was the presence of virus-specific immunoglobulin M (IgM) in umbilical cord sera. Recent virological and serological studies have shown that BKV infection does occur in pregnant women but have not confirmed the observation of congenital transmission of BKV (1, 2, 11).

Primary JCV infection is common in childhood and is as yet unrelated to any clinical illness (8). The virus is frequently identified in the urine of transplant patients (5) and of pregnant women (2) and is the etiological agent of progressive multifocal leukoencephalopathy, a rare demyelinating disease of the nervous system. We report here the results of a serological study of maternal and umbilical cord sera for JCV infection.

The sources of the sera and the donor populations have been described previously (11). One hundred pairs of maternal sera were examined. They were part of the collections made during 1959-1965 for the nation-wide Collaborative Perinatal Project (10). As a rule, the first serum tested was collected at the time of the first prenatal visit and the second was collected at the time of childbirth. The time interval between the two specimens ranged from 4 to 38 weeks with a mean of 26 weeks. Umbilical cord sera were available from six infants born to the mothers of the test group who exhibited a fourfold or greater rise to JCV antibody titers during pregnancy. These sera and an additional 300 umbilical cord sera from infants born at the University Hospital, Birmingham, Ala. (13) were tested for the presence of JCV-specific IgM.

JCV adapted to grow on primary human amnion cells (12) (kindly supplied by K. K. Takemoto) was used to prepare a virus pool which also served as the hemagglutinating antigen. All of the sera were acetone extracted and titrated in hemagglutination-inhibition (HAI) tests (11) against 4 to 8 U of JCV hemagglutinin. Both specimens from an individual were examined in the same test, and all serum pairs showing a rise in antibody titers were retested. The neutralization test has been shown to be more sensitive than the HAI test for the detection of BKV antibodies (11). Therefore, the maternal sera from early pregnancy (first specimens) were also screened for JCV-neutralizing antibodies by a simple modified neutralization test. Equal amounts of a 1:5 dilution of inactivated serum (50°C for 30 min) and a 1:10 dilution of JCV stock virus were mixed and incubated at room temperature for 1 h. Then the serum-virus mixture was inoculated onto primary human amnion cells grown in eight-well Lab-Tek slides, with one well used for each serum-virus mixture. After 7 days, the cells were harvested and tested for JCV antigen in indirect immunofluorescence (IF) tests by using a reference JCV-positive rabbit serum. A serum was considered protective if it reduced the number of cells showing JCV-specific IF by at least 90% when compared with the control. The results of simultaneous virus titration in Lab-Tek slides showed that the effective test dose was about 100 50% tissue culture infective doses. Amnion cells infected with JCV and harvested at 7 days were used as antigen for

the indirect IF test for JCV-specific IgM. The specificity and sensitivity of detection of IgM is increased when the tests are performed without the presence of competing IgG (3, 4, 9). Sera were therefore tested for JCV-specific IgM either by HAI tests of IgM fractions of the sera (separated by sucrose density gradient centrifugation) or by indirect IF tests for IgM of sera which were depleted of IgG by treatment with anti-gamma Fc (3). The effectiveness of these procedures was monitored by radial immunodiffusion tests of IgM fractions and by inclusion of sera with JCV-specific IgM in the tests.

JCV HAI antibodies were detected in 53 of the 100 maternal sera of early pregnancy (Table 1). The titers of positive sera ranged from 1:20 to 1:640 with a median value of 1:80. Neutralizing antibodies were demonstrated in all of 42 sera with HAI antibody titers of 1:40 or greater, in 9 of 11 sera with a titer of 1:20, and in 4 of 47 sera which were negative in HAI tests. Of the 100 women in early pregnancy, 43 were negative for JCV antibodies by both tests.

A 4- to 16-fold rise in titer was found in 9 of the 100 maternal serum pairs for HAI antibodies. On repeat tests, the rise in titer was reproducible. Each of the 9 mothers had both HAI and neutralizing antibodies in her first serum specimen, indicating that the rise in JCV antibody titers was a result of virus reactivation rather than of primary infection (Table 2). None of the 18 sera from the nine mothers had JCV HAI antibodies in their IgM fractions. JCV-specific HAI antibodies were also not demonstrable in the IgM fractions of umbilical cord sera of infants born to six of these mothers. In HAI tests of the additional 300 umbilical cord sera, 158 of these sera had titers of 1:80 or above; all of these 158 sera were tested for JCV-specific IgM (Table 3). The IgM fractions of the 40 sera with an HAI antibody titer of 1:1280 were examined for JCV antibodies by HAI tests as well as by IF tests for IgM. The 118 sera with titers of 1:80 to 1:640 were treated with anti-gamma Fc and then tested for IgM IF reactivity. JCV-specific IgM was not demonstrated in any serum.

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

HAI Ab titer[a]	No. of sera	Neutralizing Ab[b]	
		Present	Absent
Neg	47	4	43
20	11	9	2
40	9	9	0
80	16	16	0
160	11	11	0
320	3	3	0
640	3	3	0

[a] Ab prevalence, 53%.
[b] Ab prevalence, 55%.

TABLE 2. *Relationship of JCV antibody rise to presence or absence of antibody in the first serum sample*

Presence (+) or absence (−) of antibodies	No. of women	No. of women with antibody rise
+[a]	57	9
−	43	0

[a] In at least one test.

TABLE 3. *Examination of umbilical cord sera for JCV-specific IgM*

HAI antibody titer	No. of sera	No. of sera with IgM
≤40	142	Not done
80	37	0
160	22	0[a]
320	30	0[a]
640	29	0[a]
1,280	40	0[b]

[a] Examined by IF test for IgM after anti-gamma Fc treatment of the sera.
[b] IgM fraction examined for JCV HAI antibodies and by IF test for IgM.

Most of the umbilical cord sera with HAI antibody titers of 1:640 or greater were also screened in IF tests for IgM without prior treatment. None reacted with the JCV antigen in infected amnion cells.

We had tested these sera for BKV activity in an earlier investigation (11). The results of the two studies correlated as follows. In early pregnancy, 95% of the women had antibodies to BKV and 57% had antibodies to JCV. A total of 14 women exhibited a fourfold or greater papovavirus antibody rise during pregnancy: 5 to BKV, 9 to JCV, and none to both. All of these were reactivations. Virus-specific IgM was detected in sera of mothers after BKV reactivation but not after JCV reactivation. No evidence of congenital transmission of either virus was found. Two limitations of these negative data are recognized. First, there was no opportunity to examine the consequences of primary infections during pregnancy. In experimental infection of mice with polyoma virus (6, 7), congenital transmission is readily demonstrated if mice are infected for the first time during pregnancy but is not demonstrable if pregnancy reactivates a previously latent infection. Second, the frequency of congenital transmission is low in many of the infections which are known to be transmitted congenitally. For example, congenital rubella syndrome occurs in less than 0.001 of all births in a nonepidemic year. Thus, an examination of 300 umbil-

ical cord sera could miss congenital transmissions occurring at that frequency.

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