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Ethnic differences in polyomavirus simian virus 40 seroprevalence among women in Houston, Texas

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Abstract

Objective—To examine the prevalence and distribution among racial/ethnic groups of polyomavirus SV40 antibodies in women in Houston, Texas.

Methods—Women in three different cohorts reflecting the evolving demographics of Houston were evaluated for frequency of SV40 antibodies using a plaque-reduction neutralization assay.

Results—Women in cohort A (enrolled 1972–1973) were 68% (145/212) African-American and 32% Caucasian; the overall frequency of SV40 neutralizing antibodies was 7%. Women in cohort B (enrolled 1975–1977) were Caucasian with an overall frequency of SV40 neutralizing antibodies of 18% (37/211). Women in cohort C (enrolled 1993–1995) were 50% (199/400) African-American, 25% Caucasian, and 25% Hispanic; the overall frequency of SV40 neutralizing antibodies was 10%. Logistic regression analysis for cohort A showed no difference in SV40 neutralizing antibodies with respect to race/ethnicity, pregnancy status, number of previous pregnancies, or history of sexually transmitted diseases. For cohort C, race/ethnicity was identified as a significant factor associated with SV40 neutralizing antibodies, with Hispanics having a seroprevalence of 23% compared to 5–6% in the other two groups ($p = 0.01$).

Conclusions—A significantly higher SV40 seroprevalence was found among Hispanics than other racial/ethnic groups in the city of Houston. Findings are compatible with a model that certain population groups potentially exposed to SV40-contaminated oral poliovaccines have maintained cycles of SV40 infections.

Introduction

Simian virus 40 (SV40) is a member of the family Polyomaviridae that establishes persistent infections in susceptible hosts.[1,2] Introduction of the virus into humans is linked to the development and distribution of early forms of the poliovaccine.[3–8] Both inactivated and live attenuated preparations of the poliovaccine were produced using primary rhesus monkey kidney cells, some of which were naturally infected with SV40. Infectious SV40

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survived the vaccine inactivation treatments, and data indicate that some children and young adults in the United States likely were administered SV40-contaminated poliovaccines from 1955 through 1962.[3] Precise records do not exist, but it is believed that the distribution of SV40-contaminated inactivated poliovaccine lots varied by state in the United States and that Texas was estimated to have received lots with a low level of contamination.[9] SV40-contaminated candidate live attenuated oral poliovaccines were tested in large field trials outside the United States prior to licensing, especially from 1958 to 1960.[5] Both inactivated and live attenuated SV40-contaminated poliovaccines were administered in several countries in Latin America and other regions of the world.[5,6,10,11]

The transmission, pathogenesis, and current prevalence of SV40 infections in humans are largely unknown, but it appears that infections have occurred in target populations in different geographic regions.[12–16] Studies with enzyme-linked immunosorbent assays (ELISAs) using virus-like particles have estimated SV40 seroprevalences of 2–10%,[17–19] based primarily on serum samples obtained from the United States and the United Kingdom, both highly developed countries. SV40 seroprevalences estimated by neutralization assays have similarly ranged from 2% to 10%, with some population groups reaching 16%.[13–15,18,20–22] An immunoassay based on SV40-specific peptides from the viral capsid proteins detected SV40-specific antibodies in 18% of Italian blood donors.[23] The polyomaviruses JCV, BKV and SV40 can be differentiated serologically by hemagglutination and neutralization assays,[24] with neutralization assays based on abrogation of virus infectivity recognized as a highly specific measure of virus antibodies.[25]

While some SV40 infections in humans are related to direct exposure to early forms of the contaminated poliovaccines, markers of infection have been detected in individuals too young to have been exposed to the contaminated vaccines.[4,5] This suggests there are other sources of exposure to the virus. In addition to the recognized excretion of polyomaviruses in urine, polyomaviruses are found in human stool samples,[26–30] in sewage,[31] and in human feces-contaminated waters,[32,33] highlighting the potential for fecal–oral transmission by these agents. It was shown previously that 19% of newborn children and 15% of infants 3- to 6-months-old in the United States at the time of receiving the original contaminated oral poliovaccine excreted infectious SV40 in their stools for up to 5 weeks after vaccination.[34] Maternal–infant transmission has also been shown to be a possible route of transmission in animal models.[35] SV40 DNA has been detected in the blood in numerous studies, suggesting a possible mode of spread within a host.[36–46] A model has been proposed that SV40 human infections were established primarily by the use of contaminated live oral poliovaccines and that infections persist today in regions where living conditions allow transmission of virus by a fecal/urine–oral route.[5]

This investigation was designed to explore the prevalence of SV40 neutralizing antibodies and to seek insights into parameters of infections in three distinctive cohorts of women in Houston, Texas, one of the largest and more ethnically diverse cities in the United States. The cohorts reflect the evolving demographics of this large metropolitan area and allowed an assessment of whether race/ethnicity, year of birth, pregnancy status, number of previous pregnancies, or history of sexually transmitted diseases is associated with infections by polyomavirus SV40. A comparison of members of one cohort with mothers of the cohort addressed potential familial transmission of the virus.

Methods

Subjects and study criteria

Cohort A in this study included women enrolled between 1972 and 1973 to assess seroepidemiologic features of herpes simplex virus type 2 (HSV-2) infections and

pregnancy, as part of a study of HSV-2 associations with cervical cancer.[47] Women in cohort B were enrolled between 1975 and 1977 to monitor adverse outcomes in women exposed to diethylstilbestrol in utero.[48,49] Mothers of subjects in cohort B were included to compare SV40 infections in mothers and children. Women in cohort C were enrolled between 1993 and 1995 for a study of human papillomavirus and cervical carcinoma.[50] Women from cohorts A and C were recruited from patients of the public hospital that provides care for the uninsured and indigent of Harris County, Texas. At the time of enrollment, subjects signed informed consent and provided information related to demographics, pregnancy status, number of previous pregnancies, and history of sexually transmitted diseases (STD). The subjects self-identified their race/ethnicity from options listed on the questionnaire. Women from cohort B were recruited from private physician practices caring for middle and upper-middle class patients in Harris County. At the time of enrollment, subjects signed informed consent. Blood samples were collected from participants of all three cohorts at the time of enrollment and sera were stored at −20 °C. Only women with available archival serum samples were included in the present study; the serum bank had been moved several times and some sera could not be located. Women were designated to have been potentially exposed to SV40-contaminated poliovaccines if born before December 31, 1962 and nonexposed if born on or after January 1, 1963.

Serum neutralization assay

SV40 seroprevalence was determined using a specific plaque-reduction neutralization assay that has been described.[13,14,20] Briefly, heat-inactivated serum samples were diluted in Tris-buffered saline (TBS; pH 7.4) and mixed with equal volumes of SV40 diluted to contain 50 to 100 plaque-forming units per 0.1 ml. The virus–serum mixtures were incubated at 37 °C for 30 minutes prior to inoculation onto confluent TC7 cell monolayers. Each assay included the following controls: virus only (virus mixed with TBS), normal serum control (virus mixed with normal serum lacking SV40 antibodies), positive serum control (virus mixed with hyperimmune rabbit serum with neutralizing activity against SV40), and cell controls (TBS only). Each human serum sample was tested in triplicate. Initial screening assays involved a final serum dilution of 1:10; positive samples that reduced the number of plaques by 50% or more as compared with the virus-only control were titered in repeat experiments. Previous characterizations of the SV40 neutralization test showed there was a lack of correlation between SV40 seropositivity and BKV antibody titers[20,22] and that BKV and JCV hyperimmune rabbit sera failed to neutralize SV40.[20]

Statistical analysis

A logistic regression model was performed on cohorts A and C to determine whether the proportion of women infected with polyomavirus SV40 differed by race/ethnicity, year of birth (before 1963 vs. 1963 or later), pregnancy status, number of previous pregnancies, or history of sexually transmitted diseases. An analysis of variance (ANOVA) model was used to compare the SV40 antibody titers of women in each cohort by race/ethnicity, year of birth, and pregnancy. The statistical analysis was conducted in logarithm base 2 scales ($\log_2$ transformed) to reduce the skewness of the titers and to improve variance estimation. The logistic and ANOVA analyses were performed separately for each cohort. An ANOVA procedure assuming unequal variance was conducted to compare the antibody titers ($\log_2$ transformed) of the mothers and daughters of Cohort B. The analyses for cohort C were age-adjusted. Statistical differences were declared based on the traditional statistical significance level of 5%. All statistical analyses were performed using the statistical software SAS version 9.1.

Results

Ethnic differences in SV40 seroprevalence

The demographic characteristics and SV40 seropositivity for women in cohort A (1970s), cohort B (1970s), and cohort C (1990s) are shown in Table 1. Among the 212 women in cohort A, 68% were African-American and 32% Caucasian. Seventy-two percent of the participants were pregnant at the time of serum sample collection for the study. The overall frequency of SV40 neutralizing antibodies in this group was 7% (15/212). In cohort B, all 211 women were Caucasian and none was pregnant at the time of serum collection. The overall frequency of SV40 neutralizing antibodies in this group was 18% (37/211). Of the 400 subjects in cohort C, 50% of the women were African-American, 25% Caucasian, and 25% Hispanic. None of the women in this group was pregnant at the time of serum collection. The overall frequency of SV40 neutralizing antibodies in this cohort was 10% (39/400).

The logistic regression analysis for cohort A showed there was no significant difference in the detection of SV40 neutralizing antibodies in females with respect to race/ethnicity (p = 0.17), pregnancy status (p = 0.74), number of previous pregnancies (p = 0.81), or history of STD (p = 0.96) (Table 1). However, in cohort C, race/ethnicity was identified as a significant factor associated with the presence of SV40 neutralizing antibodies. The frequency of SV40 seropositivity was higher in Hispanic women (23/101, 23%) compared with the African-American (11/199, 6%) and Caucasian (5/100, 5%) subjects (p = 0.01). There was no significant difference in seropositivity with regard to year of birth (p = 0.76), number of previous pregnancies (p = 0.70), or history of STD (p = 0.65) for cohort C.

The mean titers of neutralizing antibodies in SV40-seropositive women for the three cohorts by racial/ethnic groups, year of birth and pregnancy status are presented in Table 2. The overall mean ± standard deviation ($\log_2$ transformed) antibody titer was 5.65 ± 1.71 (range 3.32–8.96) for cohort A, 5.83 ± 2.06 (4.32–11.32) for cohort B, and 4.97 ± 1.73 (range 3.32–10.32) for cohort C. No statistically significant differences in mean SV40 antibody titer were observed by racial/ethnic group (cohorts A and C) or year of birth (before 1963 vs. 1963 or later [Cohort C]). There was no statistically significant difference in the mean SV40 neutralizing antibody titer between pregnant and nonpregnant women in cohort A.

The majority of SV40-seropositive Hispanic women (16/23, 70%) and African-American women (7/11, 63%) in cohort C were born after 1963, in contrast to Caucasians among whom few SV40-seropositive subjects were born after 1963 (1/5, 20%). This suggests there must have been sources of exposure to SV40 among the Hispanic and African-American groups other than direct exposure to contaminated vaccines.

SV40 seroprevalence in members of cohort B and their mothers

An analysis was carried out on cohort B subjects from the diethylstilbestrol study and their mothers whose serum samples were available to compare the frequency of SV40 antibodies in mothers and children (Table 3). (Mother–daughter pairs had been enrolled in the diethylstilbestrol study.) Cohort B subjects were born between 1955 and 1962 and all were potentially exposed to SV40-contaminated vaccines. As poliovaccines were targeted to infants and children, it is unlikely the mothers of cohort B subjects would have been vaccinated. Among the 211 cohort B subjects (daughters), 37 (18%) were positive for SV40 neutralizing antibody. Serum samples were available from 72 mothers of cohort B subjects; 7 (10%) were SV40 antibody-positive. The mothers' mean ± standard deviation ($\log_2$ transformed) antibody titer was 4.03 ± 0.44 (3.32–4.32) compared to 5.83 ± 2.06 (4.32–11.32) for the daughters ($p < 0.0001$). Among those seven antibody-positive mothers, one was matched with an SV40 antibody-positive daughter; and six were matched to SV40

antibody-negative daughters. Of those latter six SV40-positive mothers, four had other children of an age to have potentially been vaccinated with an SV40-contaminated vaccine. However, no sera were available from the siblings of the daughters in cohort B to test their seropositivity. These results suggest that mothers could have been infected by contact with vaccinated infants or children.

Discussion

This investigation provides evidence of polyomavirus SV40 infections in women in Houston, Texas, in the United States. The frequency of SV40 neutralizing antibodies in Caucasians and African-Americans sampled in the 1990s was low (5–6%), similar to other reported studies from the United States.[18,19] The higher prevalence of SV40 neutralizing antibodies in Hispanic women (23%) is of interest. The city of Houston has a large Hispanic population.[51] This population is composed of immigrants or children of immigrants from Mexico, Central America, and other countries in Latin America. Data indicate that SV40-contaminated poliovaccines were administered in several of those countries to infants and young children, many of whom received pre-licensure, live attenuated, oral poliovaccines (OPV).[10,11] This is an important observation as the contaminated live attenuated vaccines contained higher levels of infectious SV40 than did the contaminated inactivated vaccine (IPV).[5,52] It is estimated that each OPV lot contained approximately 10^4–10^6 infectious units of SV40 per ml, whereas about 30% of the killed vaccine lots were contaminated and contained approximately 10^2–10^3 infectious units of SV40 per ml that had escaped inactivation.[3] A higher inoculum of SV40 to vaccinees by OPV would have increased the likelihood of infections being established. Also, the oral delivery of OPV was a more natural route of exposure than intramuscular injection of IPV. Reports indicate that in 1958–1959 in Mexico 308,000 children, including over 160,000 less than 5 years of age, were administered live attenuated Sabin poliovaccines from lots prepared in 1956.[53–55] In Costa Rica, a country in Central America, a nationwide campaign was initiated in March 1959 to vaccinate all children under 11 years of age with live attenuated poliovirus vaccine.[56] The vaccine was supplied by the United States (Lederle Laboratories). By April 1960, 71% of the estimated total population of 382,905 children under age 11 years in Costa Rica had been vaccinated. Nicaragua, another country in Central America, had its vaccination program with the live attenuated poliovaccine started in September 1958.[57] Mass vaccination was followed by a maintenance program continued throughout 1959 and 1960. By April 1960 a total of 73,533 children under 10 years of age, including 5,344 newborns, had received the OPV in Nicaragua. Similar campaigns with the live attenuated poliovaccine were conducted in South America, including Brazil, Colombia and Uruguay.[10,11,58] Therefore, the administration of SV40-contaminated OPV during large field trials in Latin America may have seeded SV40 into segments of those populations. Conditions of crowding and poor sanitation in some locations may have allowed maintenance of SV40 human infections by fecal–oral transmission that continue today.[5] It is possible that some immigrants from those regions took chronic SV40 infections with them when they migrated to Houston.

Several lines of evidence support the premise that SV40 infections are distributed unevenly and that persons of Hispanic origin are among populations that are infected. Fecal excretion of SV40 was detected in 8/99 (8%) hospitalized children in Houston.[26] Whereas Hispanics were 41% of the children studied, six of the eight (75%) excretor children were Hispanic, three of whom were immune compromised. In a follow-up study of polyomavirus shedding in the stool of healthy adults, 2/110 (1.8%) were excreting SV40.[28] Both SV40 shedders were Hispanic males (Hispanics represented 34% of the study group). In a seroprevalence study among HIV-infected men in Houston in the early 1990s, overall 38/236 (16%) had SV40 neutralizing antibodies with seropositivity among the Hispanic participants being

higher (5/19, 26%).[22] A study of children seen as inpatients or outpatients at Texas Children's Hospital in Houston found an overall SV40 seroprevalence of 6%, with seropositivity increasing with age from 1% for children less than 5 years of age to 9% in those 10–15 years old ($p = 0.01$).[20] Antibody positivity among the Hispanic children in that study was 5/85 (6%). In addition, studies of SV40 molecular markers in lymphomas from adult HIV-infected and -uninfected patients showed frequent detection of DNA and T-antigen in malignancies from Hispanic patients.[4,5,59,60] Samples from patients of the public hospital were more frequently SV40 positive (10/44, 23%) than those from the veteran's hospital in Houston (4/127, 3%) ($p < 0.0001$).[59] Five of the SV40 DNA-positive lymphomas were from Hispanic patients (5/10, 50%). An earlier study of lymphomas from HIV-positive patients obtained from the public hospital detected SV40 DNA and T-antigen expression in 12/55 (22%) lymphomas and 0/25 (0%) controls ($p = 0.01$). The 12 HIV-infected patients with SV40-positive lymphomas included 4 Hispanics.[60]

The women in cohort B had a higher seropositivity rate (18%) than did the Caucasian women in cohort A (3%), both groups of which were enrolled in the 1970s. A likely explanation is that the members of cohort B were of middle/upper-middle socioeconomic class and may have had higher polio vaccination rates than the lower socioeconomic class members of cohort A, thereby increasing their potential exposure to contaminating SV40.

It is of interest that 10% of the mothers of subjects in cohort B were found to be SV40 seropositive. As they were of an age unlikely to have been vaccinated with SV40-contaminated vaccines, this observation suggests they may have been secondarily infected by contact with vaccinated infants or children. This is reminiscent of the results from a study in Hungary and the Czech Republic, both Central European countries with well-documented, nation-wide early poliovirus vaccination programs.[14] The overall seroprevalence rates were 2–3% for Hungary and the Czech Republic but females had a higher rate of SV40 neutralizing antibodies than males in both countries, reaching 8–16% in certain age groups. It was suggested that the more frequent seropositivity in women might have been due to exposure to SV40 shed by their vaccinated children or by other young vaccinees at places of employment, such as nurseries or kindergartens.

There is limited knowledge of the human immune response to SV40. Reported neutralizing antibody titers determined in plaque-reduction or microwell neutralization assays are usually low (≤1:20), although some individuals display titers of ≥1:320.[13–15,18,20,22] In this study, antibody titers for the mothers of subjects in cohort B were similarly low (≤1:20), whereas the members of cohorts A and B tended to have higher titers (Tables 2 and 3). This may suggest that some of those exposed to a high dose of virus from the vaccine had more robust infections leading to higher SV40 neutralizing antibody titers than some individuals infected naturally.

There are limitations to this study. Information is not available on the country of origin for the Hispanic subjects, there are no records of vaccination histories for the subjects, and it is unknown how many times the test sera may have been thawed over the 17–38 years of storage with possible effects on antibody titers. Nevertheless, this analysis suggests that studies involving defined target populations are likely to identify and characterize individuals involved in the transmission of SV40 today. This would be in keeping with the Institute of Medicine recommendation for further studies of the transmissibility of SV40 in humans.[6] Populations with known contemporary SV40 infections would be targets for studies of potential virus–disease associations. Hispanics in the United States have higher incidence rates for certain cancers than do non-Hispanic whites, but the basis for those differences in the highly diverse Hispanic population is not known.[61]

In conclusion, this study showed that SV40 infections are occurring in women of childbearing age in Houston, Texas, and that members of the Hispanic population appear to have a higher frequency of infection than others. Further studies are needed to establish the distribution of SV40 infections today, determine health consequences of those infections, examine transmission pathways of polyomavirus SV40 in humans, and develop strategies to interrupt that transmission.

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Table 1

Demographic characteristics and variables included in the logistic regression analysis of SV40 seropositivity among women in Houston, Texas

Characteristics	Total subjects (%)	No. SV40 antibody-negative	No. SV40 antibody-positive (%)	Logistic regression P-value
Cohort A (1970s)				
Age, mean (± sd [range]), years	19 (± 3.5 [13–34])	19 (± 3.5 [13–34])	18 (± 3.1 [13–25])	
Year of Birth				NA
Before 1963	212	197	15 (7%)	
1963 or later	0	0	0	
Race/Ethnicity				0.17
African-American	145 (68%)	132	13 (9%)	
Caucasian	67 (32%)	65	2 (3%)	
Pregnant at enrollment[a]				0.74
Yes	150 (72%)	140	10 (7%)	
No	57 (28%)	52	5 (9%)	
No. previous pregnancies[a]				0.81
0	22 (11%)	20	2 (9%)	
1	167 (81%)	156	11 (7%)	
≥2	18 (9%)	16	2 (11%)	
History of STD[a]				0.96
Yes	24 (12%)	22	2 (8%)	
No	183 (88%)	170	13 (7%)	
Cohort B (1970s)				
Age, mean (± sd [range]), years	18 (± 2.3 [13–24])	18 (± 2.4 [13–24])	18 (± 2.0 [13–24])	
Year of Birth				NA
Before 1963	211	174	37 (18%)	
1963 or later	0	0	0	
Race/Ethnicity				NA
African-American	0 (0%)	0	0	
Caucasian	211 (100%)	174	37 (18%)	
Pregnant at enrollment				NA
Yes	0 (0%)	0	0	
No	211 (100%)	174	37 (18%)	
Cohort C (1990s)				
Age, mean (± sd [range]), years	28 (± 10.1 [15–75])	28 (± 10.0 [15–75])	30 (± 11.1 [16–65])	0.43
Year of Birth				0.76
Before 1963	144 (36%)	129	15 (10%)	
1963 or later	256 (64%)	232	24 (9%)	
Race/Ethnicity				0.01

Characteristics	Total subjects (%)	No. SV40 antibody-negative	No. SV40 antibody-positive (%)	Logistic regression P-value
African-American	199 (50%)	188	11 (6%)	
Caucasian	100 (25%)	95	5 (5%)	
Hispanic	101 (25%)	78	23 (23%)	
Pregnant at enrollment				NA
Yes	0 (0%)	NA	NA	
No	400 (100%)	NA	NA	
No. previous pregnancies[a]				0.70
0	83 (22%)	79	4 (5%)	
1	90 (24%)	84	6 (7%)	
≥2	200 (54%)	166	34 (17%)	
History of STD[a]				0.65
Yes	167 (45%)	158	9 (5%)	
No	204 (55%)	180	24 (12%)	

NA, not applicable; sd, standard deviation; STD, sexually transmitted disease.

[a]Information not available for all subjects.

Table 2

Neutralizing antibody titers[a] among SV40-seropositive women by ethnic group, year of birth, and pregnancy status

Characteristic	Cohort A		Cohort B	Cohort C		
	African-American	Caucasian	Caucasian	African-American	Caucasian	Hispanic
Total seropositive for SV40	13	2	37	11	5	23
No. born before 1963	13	2	37	4	4	7
Mean ± sd SV40 antibody titer (range)[b,c]	5.65 ± 1.71 (3.32–8.96)	7.80 ± 1.64 (6.64–8.96)	5.83 ± 2.06 (4.32–11.32)	4.57 ± 0.50 (4.32–5.32)	6.57 ± 2.87 (4.32–10.32)	4.75 ± 0.97 (3.32–5.32)
No. born 1963 or later	NA	NA	NA	7	1	16
Mean ± sd SV40 antibody titer (range)[b,c]	NA	NA	NA	4.89 ± 1.71 (3.32–7.32)	5.32	4.77 ± 1.87 (3.32–7.64)
No. pregnant	9	1	NA	NA	NA	NA
Mean ± sd SV40 antibody titer (range)[d]	5.43 ± 1.53 (3.32–8.32)	6.64	NA	NA	NA	NA
No. not pregnant	4	1	NA	NA	NA	NA
Mean ± sd SV40 antibody titer (range)[d]	5.07 ± 1.70 (3.32–7.32)	8.96	NA	NA	NA	NA

sd, standard deviation; NA, not applicable.

[a] Neutralizing antibody titers, $\log_2$ transformed.

[b] Comparison of SV40 antibody titers by ethnicity in cohort A ($p = 0.06$) and in cohort C ($p = 0.22$).

[c] Comparison of SV40 antibody titers by year of birth (before 1963 vs. 1963 or later) in cohort C ($p = 0.80$).

[d] Comparison of SV40 antibody titers by pregnancy status (pregnant vs. nonpregnant) in cohort A ($p = 0.95$).

Table 3

Analysis of SV40 seroprevalence among subjects in cohort B and their mothers

Subjects	No. tested	No. of age for potential vaccination with SV40-contaminated vaccine	No. SV40 antibody-positive (%)	SV40 antibody titer Mean ± sd (range)[a]
Cohort B (born 1955–1962)	211	211	37 (18)	5.83 ± 2.06 (4.32–11.32)
Mothers of women in cohort B[b]	72	0	7 (10)[c]	4.03 ± 0.44 (3.32–4.32)

[a] Comparison of SV40 antibody mean titers ($p < 0.0001$).

[b] Sera were not available for mothers of all women enrolled in cohort B.

[c] Among the seven SV40 antibody-positive mothers, one had a matched SV40-positive daughter and six had matched SV40-negative daughters. Four of the six antibody-positive mothers with SV40 antibody-negative daughters had other children of an age who could potentially have been vaccinated with an SV40-contaminated vaccine.