RESPONSE TO JE VACCINE AMONG HIV-INFECTED CHILDREN, BANGKOK, THAILAND

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Abstract : Since 1990, Japanese encephalitis (JE) vaccine has been part of EPI in northern Thailand, where there is a high prevalence of JE and HIV infection. To evaluate the immunogenicity and safety of JE vaccine among HIV-infected children, we conducted a retrospective study of HIV-infected and uninfected children who received 2 doses of JE vaccine at 12 months of age. Pre- and post-immunization plasma specimens were tested by plaque reduction neutralization for antibody levels to JE and dengue(1-4) viruses; titers of ≥10 were considered positive. Excluding 5 children with preimmunization antibodies, 5 of 14 (36%) HIV-infected children and 18 of 27 (67%) uninfected children had positive JE antibody titers after immunization [odds ratio (OR) 0.3, p=0.06]; 31% absolute difference [95% confidence interval (CI) 0-61.7%). The geometric mean titer of HIV-infected children with positive titers was lower than that of control children (15.1 *vs,* 23.8; p=0.17). No significant vaccine-associated adverse events were noted. We conclude that primary antibody response to JE vaccine was low among HIV-infected children and was approximately half of that seen among uninfected children. In endemic areas, HIV-infected children are likely to be at risk of acquiring JE despite routine immunization with 2 doses.

INTRODUCTION

Japanese encephalitis (JE) is the leading cause of viral encephalitis in Asia: annual reported incidence is 35,000-50,000 cases (Tsai and Yu, 1994). Reported cases in Asia exceed the estimated incidence of herpes encephalitis worldwide. Two thirds of the world population is potentially at risk for JE in Asia; highest risk is in hyperendemic areas in Southeast Asia, China, and India. JE is preventable through immunization with safe and effective vaccines. An inactivated JE vaccine has been used extensively in Japan, Taiwan, and Korea, effectively eliminating the disease in those countries. In 1990, JE vaccine was introduced into the childhood Expanded Program on Immunizations (EPI) immunization schedule in the northern provinces of Thailand, where JE is hyperendemic (Sangkawibha *et al,* 1992). Northern Thailand also has high rates of human immunodeficiency virus (HIV) infection: HIV seroprevalence among childbearing women is 5-10%, and the HIV perinatal transmission rate is 25-40% (Thisyakorn *et al,* 1994).

With the introduction of JE vaccine into the national Thai EPI schedule, many HIV-infected children will be receiving JE vaccine routinely, although their response to vaccination is not known. Lower seroconversion rates and waning immunity to a variety of childhood vaccines have been described among HIV-infected children (Borkowsky *et al,* 1987; Opravil *et al,* 1991; Palumbo *et al,* 1992; Brena *et al,* 1993; Arpadi *et al,* 1994; Chadwick *et al,* 1994; Rudy *et al,* 1994; Al-Attar *et al,* 1995; Gibb *et al,* 1995; Kale *et al,* 1995; Sibailly *et al,* 1997). However, no data are available on the safety or response rate of JE vaccine among HIV-infected children. As part of a perinatal HIV transmission study, we performed a nested retrospective study of the response to 2 doses of JE vaccine given at 12 months of age to HIV-infected and uninfected children.

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METHODS

Study population

From November 1992 through March 1994, HIV-seropositive pregnant women identified by routine antenatal HIV counseling and testing were offered enrollment into a perinatal transmission study at 2 large hospitals in Bangkok. Among 281 infants who were born during this study and whose HIV infection status was known, the HIV perinatal transmission rate was 24.2% (Shaffer *et al,* 1996). For HIV-infected infants, the 1-year survival was 82%, and the 2-year survival was 75%; for uninfected infants, the 2-year survival was 100% (Chotpitayasunondh *et al,* 1997).

At 1 of the 2 study hospitals, children were routinely given JE vaccine. Study children who had received 2 doses of JE vaccine 1-3 weeks apart at approximately 12 months of age and whose pre-immunization (at 12 months of age) and post-immunization samples (at 15-18 months of age) being available for analysis were eligible for inclusion. All eligible HIV-infected children and a 2:1 convenience sample of uninfected children were included in this JE vaccine study.

Child follow-up and HIV infection status

Study children were followed up at 1, 2, 4, 6, 9, 12, 15, and 18 months, according to the local well-child follow-up schedule. A venous blood sample (1-3 ml) was collected in an EDTA vacutainer tube at study visits at 2, 6, 12, and 15 months and processed for flow cytometry (CD4+ cell counts), HIV enzyme immunoassay (EIA) and Western blot (WB) testing, and HIV qualitative DNA polymerase chain reaction (PCR). Residual plasma was stored at -70°C. HIV infection status was determined by HIV antibody testing at 12-18 months and PCR test results at 6 months or later. All HIV-infected children were PCR-positive on at least 2 different tests and were EIA- and WB-positive at 15 months of age; all uninfected children were PCR-negative at 6 months of age and EIA- and WB-negative at 15 months of age.

Routine Vaccinations

Study children received routine childhood vaccinations according to the local EPI schedule. These included BCG at birth; hepatitis B vaccine at birth and at 1 and 6 months; oral polio vaccine (OPV) and diphtheria-pertussis-tetanus (DPT) vaccines at 2, 4, 6, and 18 months; and measles or measles-mumps-rubella (MMR) vaccine at 9 months and MMR at 15 months. At one of the study hospitals (Children's Hospital), children also routinely received JE vaccine at 12 months, followed by a 2nd dose 7-21 days later. They were also scheduled for a 3rd dose booster at 2-3 years of age (samples were not available for testing). Although JE is not considered endemic in Bangkok, many children receive the vaccine routinely because sporadic cases occur locally and children frequently travel to areas of the country where risk is higher.

JE vaccine

Various lots of mouse brain-derived inactivated JE vaccine (Nakayama strain), produced by the Thai Government Pharmaceutical Office, were administered subcutaneously according to the standard schedule of two 0.5 ml doses 1-3 weeks apart.

Laboratory testing

JE and dengue neutralizing antibody titers in plasma were determined by a modified plaque reduction neutralization test in LLC-MK_2 cells grown in 24-well plates against JE virus (Nakayama strain), dengue 1 (Hawaii), dengue 2 (New Guinea C), dengue 3 (H-87), and dengue 4 virus (H-241) (Okuno *et al,* 1978; Moren *et al,* 1985). All testing was performed on coded samples; paired samples from the same child were tested on the same run. Heat-inactivated samples were tested at 2-fold dilutions beginning at 1:10. The endpoint neutralizing plasma dilution was calculated from the dilution series by probit analysis; a 50% reduction of the plaque number in the viral input dose was taken as the endpoint (Russell *et al,* 1967).

Serial HIV EIA and WB testing and CD4+ lymphocyte subset testing were performed on samples obtained from the children every 3-6 months of age, according to standard methodologies. Whole blood lysate specimens were tested for HIV by DNA PCR to confirm the HIV status of the children.

Definition of Response to JE Vaccine

Neutralization titers of ≥1:10 (≥10) to JE or dengue viruses were considered positive. Children whose JE pre-immunization titer was <10 and whose post-immunization titer was ≥10 were considered

to have responded to the vaccine. Children with a positive titer to 1 of the 4 dengue viruses were considered to have had a natural dengue infection and possible cross-reactive antibody.

Adverse events monitoring

Data on interval outpatient medical visits and hospitalizations were recorded by the study staff at all study visits. Reports of adverse events experienced by the child related to JE vaccination were recorded as part of the medical record at the time of the 2nd vaccination and at the return study visit at 15 months.

Statistical analysis

Categorical comparisons between HIV-infected and uninfected children were based on the percentage with a positive pre-immunization titer and seroconversion from a negative to positive titer after 2 doses of JE vaccine. Additional categorical comparisons of CD4+ count and clinical signs and symptoms of AIDS were made between HIV-infected infants who seroconverted and those who did not seroconvert. Comparisons were made by chi-square or Fisher's exact test when cell values were less than 5. Comparisons between continuous values, such as time between immunizations and CD4+ count, were made by using the Wilcoxon rank sum test. For children who had a positive response to JE vaccine, geometric mean titers (GMTs) were calculated and compared by using the 2-sample Student's *t*-test. To study the effect of JE vaccine on the CD4+ counts of HIV-infected children, we compared the median difference and percentage change in CD4+ counts for the HIV-infected children who had received JE vaccine and for the HIV-infected children in the perinatal cohort at the other study hospital, where JE vaccine was not given, and for whom data were available at 12 and 15 months. All p-values are 2-sided, and p-values ≤ 0.05 were considered statistically significant.

Ethical considerations

The study protocol was approved by the Thailand Ministry of Public Health Ethical Review Committee and by the CDC Institutional Review Board. Voluntary, written informed consent was obtained from all study participants.

RESULTS

A total of 46 eligible children were included in this retrospective study of response to JE vaccine, including 15 HIV-infected children (7 males) and uninfected children (15 males). On the basis of the preselection criteria, all these children received 2 doses of JE vaccine at 12 months of age, and all had adequate pre- and post-immunization plasma samples for testing. None of these children had a clinical history suggestive of JE or dengue before this evaluation.

HIV-infected and uninfected children were similar with respect to pre-immunization JE antibody titers, the timing of immunizations, and the post-immunization evaluation (Table 1). Positive pre-immunization JE antibody titers were detected in 5 (11%) of the 46 children. The median time between the 1st and 2nd JE immunizations was 7 days in both groups and the median time between the 2nd immunization and the post-immunization sample, which was used to evaluate vaccine response, was 84 days.

Among the 14 HIV-infected and the 27 uninfected children with a negative pre-immunization JE antibody titer, 36% (5) of HIV-infected children responded to 2 doses of JE vaccine, compared with 67% (18) of the uninfected children. Although the sample was small, this was of borderline statistical significance [odds ratio (OR) 0.3; 95% CI 0.06-1.29; p=0.06]. The absolute difference in the response to JE vaccine was 31% (95% CI 0-61.7%). The response rate was not different by sex (not shown). Among those with a positive response to vaccine, the GMT seemed to be lower among HIV-infected children than among uninfected children (GMT = 15.1 *vs* 23.8; p=0.17), but the difference was not statistically significant. Three children (7%) had a single pre- or post-immunization sample that had a positive, but low, dengue 1 antibody titer, suggesting cross-reactivity (data not shown). None of the samples had antibodies to dengue 2, 3, or 4 viruses.

In Table 2, we compare JE vaccine responders and nonresponders among the HIV-infected children, with respect to immunologic and clinical factors that might explain the failure to seroconvert or respond to vaccine. CD4+ counts for HIV-

Table 1

Response to JE vaccine among HIV-infected and uninfected children.

	HIV-Infected	Uninfected	OR	p
	(N=15)	(N=31)		
Positive JE titer pre-immunizationa[a]	1/15 (7%)	4/31 (13%)	0.5	1.0
Median time between immunizations (range)	7 days (7-15 days)	7 days (6-15 days)		1.0
Median time between 2nd immunization and post-immunization sample (range)	84 days (43-97)	84 days (77-92)		1.0
Positive titer after 2 doses JE vaccine[a,b]	5/14 (36%)	18/27 (67%)	0.3	0.06
GMT (of positives)	15.1	23.8		0.17

[a] Titer ≥ 1:10.
[b] Excludes children who had a positive pre-immunization titer.

Table 2

Characteristics associated with response to JE vaccine among HIV-infected children.

Factor	Responders to vaccine		p
	Yes	No	
	(n=5)	(n=9)	
Immunologic[a]			
Median CD4 count, 12 months (range)	1,750 (830-2,840)	1,400 (540-2,900)	0.6
CD4 count >1,000, at 12 months	4/5 (80%)	6/9 (67%)	1.0
CD4 count >1,500, at 12 months	3/5 (60%)	4/9 (44%)	1.0
Median CD4 count, 15 months (range)	800 (570-2,220)	1,040 (660-1,720)	0.5
Clinical			
WHO classification at 12 months			
AIDS	2/5 (40%)	1/9 (11%)	0.5
Any major or minor sign	4/5 (80%)	8/9 (89%)	1.0
CDC AIDS-defining condition	2/5 (40%)	1/9 (11%)	0.5

[a] CD4 counts expressed as cells/mm^3.

infected children who responded to JE vaccine were slightly higher than for those who did not respond, when analyzed either as a continuous variable (median of 1750 *vs* 1,400) or as a dichotomous variable (60% *vs* 44% above 1,500). Although suggestive, the differences were not statistically sig-

nificant. The clinical status of the HIV-infected children at the time of vaccination also was analyzed as a possible covariate. The proportion of children with AIDS was slightly higher but not significantly different among the responders, and the percentage with a WHO-defined major or minor sign of HIV illness was nearly the same in both groups.

To address the question whether JE vaccine might accelerate disease progression among HIV-infected infants, we compared the HIV-infected study infants who had received JE vaccine with HIV-infected infants from the other study hospital, where JE vaccine was not routinely given (Table 3). The median CD4 counts at 12 months (pre-immunization) and at 15 months were comparable for the two groups. Among the HIV-infected children who received vaccine, the median decrease in CD4+ counts from 12 months to 15 months was 200 cells/mm^3 and the median percentage decrease in CD4+ counts was 22%. Similar decreases were seen in the HIV-infected comparison group who had not received vaccine.

Adverse events

Both doses of vaccine were well tolerated. There were no serious medical complaints, extra outpatient visits, or hospitalizations associated with either of the JE vaccine doses. There also were no reports of serious rash, fever, or local reaction at the vaccine site in either the HIV-infected or the uninfected groups.

DISCUSSION

Inactivated JE vaccine is given routinely to children at 12 months of age in many provinces of Thailand and in other JE endemic areas of Asia where HIV also is epidemic. We found that the immune response to routinely administered JE vaccine was poorer in HIV-infected infants than in uninfected control infants: among HIV-infected infants, both the proportion of vaccine responders (36% *vs* 67%) and the post-immunization GMTs among responders (15.1 *vs* 23.8) were lower. Although the differences were only of borderline

Table 3

Changes in CD4+ count among HIV-infected children in response to JE vaccine[a].

CD4 counts[b]	Received JE vaccine		p
	Yes	No	
	(n=14)	(n=21)	
Median CD4 count, 12 months (Range)	1,550 (540-2,900)	1,540 (140-3,480)	1.0
Median CD4 count, 15 months (Range)	1,030 (570-2,220)	1,265 (70-3,740)	0.9
Median CD4 count difference	-235	-180	0.8
Median CD4 count percent change	-0.22	-0.19	0.98

[a] Table compares the 14 HIV-infected children who received JE vaccine and who had negative pre-immunization titers with 21 HIV-infected children from another study hospital in the same cohort who did not receive JE vaccine (one hospital routinely administered JE vaccine, and one hospital did not).
[b] CD4 counts expressed as cells/mm^3.

statistical significance, we had access to a small sample of 14 infected and 27 control infants for this study, which was performed retrospectively as part of a perinatal HIV transmission study. Furthermore, our hypothesis was that HIV-infected children were likely to have lower antibody responses. In addition, no significant vaccine-associated adverse events were noted.

Although two JE vaccine doses produced immune response rates approaching 100% in children from northern Thailand and in other JE endemic areas (Rojanasuphot *et al,* 1992), these high response rates likely reflect a combination of anamnestic responses in subjects already exposed to dengue, JE, and other flaviviruses, as well as primary responses to the vaccine. The response rate to 2 doses of JE vaccine in our control infants, 67%, was similar to rates observed in other JE vaccine immunogenicity studies in which naturally acquired flaviviral infections that might have primed the JE vaccine immune response could be discounted (Tsai and Yu, 1994). Our study subjects resided in Bangkok, where JE rarely is transmitted; only 1 infant had a positive dengue 1 titer before vaccination. Thus, it is the lower relative proportion of vaccine responders among HIV-infected infants (OR 0.3; response difference 31%) that should be underscored.

The poor immune response of HIV-infected infants to inactivated JE virus vaccine was similar to reported experiences with live and inactivated antigens. Age at immunization, interval between immunization and post-immunization antibody determination, clinical stage, and pre-immunization CD4+ counts or CD4 to CD8 ratio have been associated with the immune responses to various vaccines. In our study, the age at vaccination and the interval between doses were tightly clustered at the recommended times, precluding analysis of this effect. Because our sample was small, we were unable to demonstrate any definite associations between clinical or immunologic markers of HIV infection and antibody response to JE vaccine. However, there was a suggestion that the CD4+ cell count was lower among HIV-infected nonresponders. On the other hand, we could detect no major adverse effect of JE vaccination on this laboratory indicator of HIV progression. Compared with unvaccinated HIV-infected infants, vaccinated infants had a similar decline in CD4+ cell counts between 12 and 15 months of age. Markers of viral burden, such as plasma HIV RNA, were not measured.

The few reported studies of immune responses to other flaviviral vaccines in HIV-infected subjects have produced mixed results. In a study of HIV-infected adults who had CD4+ counts of >200/mm^3 and who had received live-attenuated yellow fever vaccine, 87% had immune responses (Goujon *et al,* 1995). In a study of HIV-infected African infants, however, only 17% of the 1-year old infants who received yellow fever vaccine responded (Sibailly *et al,* 1997). No significant adverse events after vaccination were noted in either study. The reasons for these widely discrepant response rates are unknown, but the differences could be related to degree of immunocompromise, age, or other factors. Immune responses to another flaviviral vaccine was reported for 4 HIV-infected hemophiliac patients who had received inactivated tickborne encephalitis vaccine. All 4 responded to the vaccine but, compared with controls, produced significantly lower antibody levels and did not produce a T-cell proliferative response (Wolf *et al,* 1992). Because the various contributions of humoral and cellular immunity to long-term protection against JE and other flaviviral infections are only partially understood, observations from our study and earlier studies suggest caution in assuming protection in HIV-infected persons who receive these vaccines.

Although mortality among HIV-infected infants in Thailand is approximately 20% during the 1st year of life, median survival for the remaining children is estimated at 5 years (Chotpitayasunondh *et al,* 1997). In Thailand, as elsewhere in Asia, HIV-infected children who survive beyond the first several years of life will be at risk of acquiring JE, the leading identifiable cause of childhood viral encephalitis in the region. In the pre-immunization era, JE was responsible for thousands of encephalitis cases each year in Thailand (10-20% fatal); incidence rates exceeded 5/100,000 in highly endemic areas in the north. Age-specific incidence remains highest for children 5 to 9 years of age in Thailand but peaks at an earlier age – 2 to 5 years of age – in China.

Increasing numbers of adults and infants with HIV-infection in Asia are exposed to indigenous infectious agents, including arboviruses such as JE. However, few reports have described the clinical manifestations of arboviral infections in AIDS patients or HIV-infected persons. In a St Louis en-

cephalitis outbreak in Houston, Texas, a greater proportion of epidemic cases occurred in HIV-infected persons than would have been expected in the exposed population (Okhuysen *et al,* 1993). In a later outbreak, anecdotal observations did not suggest a more aggressive clinical course in HIV-infected adults (J Luby, 1997 unpublished observations). The clinical manifestations of JE, dengue, or other flaviviral infections in HIV-infected or other immunocompromised children or adults is unknown.

As the HIV epidemic in Asia spreads, its geographic range will overlap with those of arboviruses and other regionally endemic diseases. Additional studies are needed to determine how best to use existing vaccines to protect HIV-infected children from common serious infections such as JE that are preventable in non-immunocompromised children.

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