Measurement of Antibodies to Varicella-Zoster Virus in a Tropical Population by Enzyme-Linked Immunosorbent Assay

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We used the recently developed enzyme-linked immunosorbent assay for antibody to varicella-zoster virus to study the prevalence and titers of virus-specific antibody in a south Indian population of 171 individuals 0 to 25 years old. The antibody prevalence rate was less than 15% in individuals under 5 years of age and gradually rose to a maximum of 72% in young adults 15 to 25 years of age. The median age of primary infection was 12.25 years. The geometric mean antibody titers were between 1:80 and 1:160 in the age groups with antibody. These results are different from the pattern of seroepidemiology of varicella-zoster virus infection in temperate countries and indicate that varicella is predominantly a disease of young adults in the population studied.

Varicella epidemiology is poorly understood in tropical countries. Since most children in temperate regions are immune to varicella by the age of 12 years, the disease is rare in adults (3, 8). It occurs more frequently in adults in the tropics (2, 5, 7). The prevalence of immunity to varicella-zoster virus (VZV) in the tropics has not been previously investigated. Here we report the use of the enzyme-linked immunosorbent assay (ELISA) to study the seroepidemiology of VZV infection in a tropical population. The sensitivity of the ELISA and its correlation with the virus neutralization test are excellent (1, 4). The technique is specific (4), and cross-react with herpes simplex virus is minimal (unpublished data). Moreover, it detects antibody many years after primary virus infection and serves as a reliable indicator of past VZV infection, unlike the more commonly used complement fixation method (1, 4).

Serum samples were collected from 171 individuals residing in Vellore or the surrounding rural areas who were admitted to the hospital for illnesses unrelated to varicella or zoster. They were in eight age groups (number of individuals): less than 6 months (20), 6 to 11 months (24), 1 year (21), 2 years (19), 3 years (20), 4 years (19), 5 to 14 years (24), and 15 to 25 years (24). Serum samples were stored at −20°C until they were tested.

VZV antigen was prepared from MRC-5 fibroblast cells infected with strain D507 of the virus (1). Infected cell cultures with 75 to 100% cytopathic effect were scraped, collected, and centrifuged at 600 × g for 10 min at 4°C. The cell pellet was suspended (10% [vol/vol]) in phosphate-buffered saline (pH 7.2) and disrupted by freezing at −70°C and rapid thawing to 37°C six times in succession. It was then centrifuged at 600 × g for 30 min, and the final pellet was resuspended in 2.5 ml of cold phosphate-buffered saline. Negative control antigen was prepared from uninfected MRC-5 cells treated similarly. Both preparations were stored at −70°C until used.

Optimal dilutions of the virus antigen, alkaline-phosphatase conjugated anti-human immunoglobulin G (IgG) (gamma chain specific; Behring), and similarly conjugated anti-human IgM (Mu chain specific; Behring) were determined by checkerboard titration. All serum samples were tested for IgG and IgM antibodies to VZV by a modification of the microplate ELISA method (6). Briefly, 50 μl of optimal dilutions of virus and control antigens in carbonate-bicarbonate buffer (pH 9.6) were placed in alternate rows of the wells of micro-ELISA plates (Dynatech M129 B) and incubated overnight at 4°C. After being washed three times in phosphate-buffered saline with 1% Tween 20, the sensitized plates were dried and stored at 4°C until use. Two hundred microliters of a single dilution of serum (1:10 for IgM and 1:40 for IgG determinations) was tested in parallel against VZV and control antigens. After incubation at 37°C for 3 h, the plates were washed three times in phosphate-buffered saline with 1% Tween 20, and 50 μl of optimal dilutions of the appropriate conjugates was added to each well. The plates were washed after 1 h at 37°C, and 100 μl of substrate solution (1 mg of sodium p-nitrophenyl phosphate [Sigma 104] per ml in 9.7% diethanolamine buffer [pH 9.8] with 4.8 mM MgCl2·6H2O) was added to each well. After incubation at 25°C for 45 min, 25 μl of 2 N sodium hydroxide was added to each well and absorbance was read.

![FIG. 1. Age-specific prevalence rates of IgG antibodies to varicella-zoster virus.](http://jcm.asm.org/)

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added to each well. Reactions were read at 405 nm with a plate-reading photometer (Titertek Multiskan; Flow Laboratories), and virus-specific activity was calculated from the difference between VZV and control antigen wells. Antibody titers were then determined from nomograms constructed by titration of reference reagents. Sera with antibody titers ≥1:40 dilution (for IgG) or ≥1:10 dilution (for IgM) were defined as positive.

The age-specific prevalence rates and geometric mean titers of IgG antibodies to VZV are shown in Fig. 1 and 2. No serum was positive for virus-specific IgM antibody. Only 25% of children had antibody in the first 6 months of life, presumably maternal in origin. No child had antibody during the second year. The rates gradually increased thereafter, with the highest prevalence (72%) in the 15- to 25-year age group. The geometric mean titers of IgG antibodies to VZV were between 1:80 and 1:160 in all age groups with antibodies.

A trend line depicting the rate of change was mathematically fitted to the values of age-specific antibody prevalence rates, and the median age for primary VZV infection was found to be 12.25 years.

This pattern of age-specific prevalence of antibodies to VZV is different in several ways from that in temperate countries, where most 12-year-olds are immune to varicella (3, 8). First, the median age of primary infection is delayed. Second, the antibody prevalence rate (72%) in individuals 15 to 25 years old is lower than that in temperate countries (1, 7, 9). In contrast, in France 93% of healthy, 20- to 25-year-old blood donors were seropositive by the standardized ELISA used in this study (unpublished data). Third, the geometric mean titer of VZV-specific antibody (1:42) in French blood donors was lower than that (1:98) of the 15- to 25-year-old group in this study, suggesting that primary infection occurs at an older age in India. Together, these results provide serological evidence that varicella epidemiology in this population is different from that in temperate areas.

It has been suggested that varicella is hemagglutinin in the tropics because of the rural, discontinuous nature of the population or because the transmission potential of this relatively heat-labile virus is decreased by high ambient temperatures in the tropics (6). Our communities are overcrowded rather than discontinuous, and we favor the latter explanation. Evidence in support of this possibility has been presented in an earlier report (A. R. Venkitaraman and T. J. John, Int. J. Epidemiol., in press).

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LITERATURE CITED