Establishment by Enzyme-Linked Immunosorbent Assay of Seronegative Range for Herpes Simplex Virus and Cytomegalovirus Antibodies and Evaluation of Heterologous Responses to Live Varicella Vaccine

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Cord serum samples treated with staphylococcus protein A and an immune affinity column to remove immunoglobulin G were used to establish a seronegative range for herpes simplex virus and cytomegalovirus.

No seroconversions or increased heterologous antibody levels to herpes simplex virus or cytomegalovirus were found in live varicella vaccine recipients.

To determine whether an individual is susceptible to an organism or whether the individual has had a seroconversion following infection or immunization, seronegativity must first be defined. This has been done previously for varicellazoster virus (VZV) by assaying serum samples obtained from patients prior to onset of varicella (13). Because clinical manifestations of primary herpes simplex virus (HSV) or postnatal cytomegalovirus (CMV) infection are often not detectable, such an assay was not feasible for these viruses. A defined seronegative range would allow assessment of whether immunization with live varicella vaccine (LVV) (15) would result in increased heterologous antibody to HSV or CMV. HSV infections have been shown to elicit increased heterologous antibody to HSV (5, 9, 12). HSV glycoprotein B and the 63,000-dalton VZV glycoprotein (gp63) share antigenic epitopes (4). Homologous sequences have been identified in the genes coding for the glycoproteins of different herpesviruses (2).

Increased heterologous antibody would suggest that LVV might confer some protection against HSV and CMV. To study this, serum samples obtained from children before and following administration of LVV were tested for antibody titers to HSV and CMV, and the seroconversion rates were determined.

Antigens for enzyme-linked immunosorbent assay were produced from the KOS strain of HSV type 1, obtained from K. Smith; the Ellen strain of VZV, isolated by P.A.B.; and CMV AD-169 (ATCC VR-538).

HSV, VZV, CMV, and control antigens were prepared as previously described for varicella antigen (13). The standardization and performance of the enzyme-linked immunosorbent assay were as described previously (3).

Since antibody in cord serum is derived primarily from transfer of maternal immunoglobulin G (IgG) (8), the seronegative range was estimated for HSV and CMV by testing five cord serum samples that were treated with an IgM-IgG isolation system II (Isolab; Akron, Ohio) (14) to remove IgM, IgG1, IgG2, and IgG3 and with staphylococcus protein A (Calbiochem-Behring, La Jolla, Calif.) (7) to remove IgG1, IgG2, and IgG4.

A serological survey for antibody against CMV and HSV was performed by studying serum samples from 273 and 279 individuals, respectively. The samples included randomly obtained cord blood samples from newborns at Medical Center Hospital in San Antonio, Tex.; samples from infants aged 12, 15, and 18 months who had not received LVV; and samples from volunteer medical students.

For the study of increased heterologous antibody in LVV recipients, blood was obtained from 43 children before immunization and 3 months after immunization. An age-matched control group of 36 children whose blood was drawn at similar intervals was tested to determine rates of seroconversion to HSV and CMV in the absence of vaccine.

A single serum sample positive for HSV type 1 or CMV by immunofluorescence and two samples positive for VZV by enzyme-linked immunosorbent assay were included in multiple tests designed to assess reproducibility. One lot of antigens and two different lots of conjugate were used in the tests. The variability in delta optical density (OD) readings (mean OD at 405 nm of two viral wells minus two control

FIG. 1. Serological survey for CMV antibody levels in 273 individuals. The shaded area represents 3 standard deviations above the mean for the treated cord sera. The mean (± standard deviation) delta OD levels for the treated cord sera were not significantly different from the lower cluster of values for the 12- and 15-month-old infants.

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from the wells) was 15% or less. In no case was there a change in immune status.

The serological surveys revealed bimodal peaks for both CMV and HSV (Fig. 1 and 2). The means and standard deviations for the treated cord sera and for the lower peaks at 12 and 15 months were, respectively, as follows: for HSV, 0.037 ± 0.018, 0.027 ± 0.022, and 0.013 ± 0.011 (not significant); and for CMV, 0.006 ± 0.022, 0.011 ± 0.026, and 0.019 ± 0.026 (not significant). If the higher values can be assumed to represent seropositivity, then the percentage of immune individuals was greater in the newborns and in the adults than in the infants (Tables 1 and 2).

After the seronegative range was defined for both antigens, the heterologous responses of 43 infants who had been vaccinated with LVV and seroconverted to VZV was studied. The delta OD for VZV before immunization was 0.012 ± 0.012, and 3 months later it was 0.187 ± 0.135. In the 36 age-matched nonimmunized infants, none were seropositive for varicella at 12 or 15 months (delta ODs, 0.012 ± 0.030 and 0.006 ± 0.025, respectively).

One seroconversion to CMV was noted in both the vaccinated and the nonvaccinated groups. Two seroconversions to HSV in the nonvaccinated and none in the vaccinated children were observed. There were no significant differences in the mean pre- and postimmunization values for CMV or HSV. All of the vaccinees were seronegative for HSV just before LVV immunization, whereas 6 of the 40 vaccinees were seropositive for CMV. None of these infants had a boost in titer after LVV.

| TABLE 1. Individual seropositivity for CMV by age group |
|-----------------------|-----------------------|
| Age       | n   | No. (%) positive* for CMV antibodies |
| Birth     | 79  | 36 (46) |
| 12 mo     | 76  | 9 (12) |
| 15 mo     | 36  | 3 (8)  |
| 18 mo     | 40  | 11 (28)|
| Adult     | 42  | 15 (36)|

* Delta OD > 0.072, calculated from 3 standard deviations above the mean for the treated cord sera.

Ideally, serum samples should be obtained before onset of disease to establish a seronegative range. Most CMV or HSV infections which occur postnatally are subclinical, and procurement of such samples would be difficult. Cord serum samples were treated with staphylococcus protein A and an immunoaffinity system to obtain IgG-free sera. Staphylococcus protein A treatment alone does not remove IgG3, and the immune affinity column fails to remove IgG4; both subclasses are prevalent in both HSV and CMV (6, 16). After treatment, the values obtained closely approximated those in the cluster of low values found in our seroprevalence studies. The seroprevalence values for CMV (17) and HSV (10) were similar to those found in infants and adults by other methods of antibody determination. The incidence of seropositive cord serum samples was similar to that found for adult samples for each antigen, reflecting that cord serum antibody is derived from maternal IgG. Finally, there appeared to be a low rate of CMV and HSV infection during the first 15 months of life, similar to that reported previously for VZV (1).

Immunization of children with LVV did not result in antibody increases to CMV or HSV, other Herpesviridae family members. Therefore, LVV would not be expected to confer protection against CMV or HSV. That heterologous antibody responses to the herpes antigens occurred in individuals who were already immune has been reported elsewhere (11). All the vaccinees were HSV negative. However, no increases in CMV antibody were found in the seropositive recipients.

LITERATURE CITED


