Serum Immunoglobulin A Antibody to Varicella-Zoster Virus in Subjects with Primary Varicella and Herpes Zoster Infections and in Immune Subjects

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Immunoglobulin A (IgA) antibodies to varicella-zoster virus (VZV) were measured in sera from subjects with acute varicella and herpes zoster, VZV-immune subjects remote from infection, and recipients of a live attenuated varicella vaccine, using a solid-phase radioimmunoassay. Primary infection with VZV was associated with early production of IgA antibodies. Among 36 subjects with varicella tested 1 to 5 days after onset, 22 had detectable IgA, and all of the negative sera were obtained before day 3 of the varicella exanthem. VZV IgA was detected in one of three sera obtained more than 60 days after onset of the illness. Four of five sera obtained from subjects within 1 week of the onset of herpes zoster had measurable levels of IgA. Between 1 and 4 weeks after onset of zoster, all 10 subjects tested had detectable IgA to VZV. VZV IgA was detected as late as 63 days after the onset of herpes zoster. Of 10 vaccine recipients, 5 developed VZV IgA which was detected as early as 4 weeks and persisted for as long as 16 weeks after vaccination. VZV IgA was not detected in sera from 42 children who had no detectable IgG antibody to VZV. VZV IgA was found on only 3 of 23 sera from adults who had varicella more than 20 years before.

Varicella-zoster virus (VZV) causes varicella, a common illness of early childhood. Primary VZV infection is usually self-limited, but dissemination and life-threatening illness may occur when the patient has underlying malignancy or immunodeficiency (7). As immunization has reduced the incidence of other childhood viral infections, varicella has become associated with a relatively greater risk of severe complications in otherwise healthy children. From 1972 to 1978, 403 cases of varicella encephalitis occurred in the United States, compared with 183 cases of measles encephalitis (15).

Recent research has focused upon the development of VZV vaccines for potential use in immunocompromised and healthy children (3, 9, 10, 12). It will be important to determine how well the immune response to these vaccines parallels immunity with natural VZV infections. Because VZV is presumed to have a respiratory mode of transmission, antibodies of the immunoglobulin A (IgA) class may be important in the host response.

The solid-phase radioimmunoassay (RIA) is a sensitive method for the detection of antibodies of the IgG and IgM classes to herpesviruses, including herpes simplex virus (HSV), cytomegalovirus, and VZV (1, 6, 8, 13). In this study, a

solid-phase RIA technique was developed to measure specific IgA antibodies to VZV.

MATERIALS AND METHODS

Subjects. Sera from 23 adults who had IgG antibody to VZV by RIA were tested for VZV IgA. These subjects had had primary varicella more than 20 years before and had no history of herpes zoster. Sera from 42 children who had no IgG antibody to VZV by RIA were assayed for VZV IgA. Sera were also tested from 37 otherwise healthy subjects with acute varicella and from 16 healthy subjects with herpes zoster. Paired sera were available from 22 subjects with acute varicella and from 6 subjects with zoster. Sera from 10 recipients of the Oka strain of live attenuated varicella vaccine were also evaluated. Seven of the vaccinees were children with hematological malignancies, and three were healthy adults. These subjects were participants in the National Institutes of Health Collaborative Study of varicella vaccine directed by Anne Gershon, New York University.

RIA for VZV IgA. The RIA for VZV IgA was a modification of the RIA for VZV IgG (1). In brief, commercial VZV complement fixation antigen or uninfected tissue control antigen (Flow Laboratories Inc., Rockville, Md.) was diluted 1:5 with phosphate-buffered saline (PBS), added to wells of polyvinyl U plates (Dynatech Co., Alexandria, Va.) (0.025 ml per well), and allowed to dry overnight. After being washed with PBS, the wells were filled with 3% bovine serum

albumin (BSA) and incubated for 1 h at 37°C. The plates were rinsed with PBS and allowed to dry. Serum specimens to be tested were diluted 1:4 with PBS containing 1.5% BSA and 0.05% Tween 20 (J. T. Baker Chemical Co., Phillipsburg, N.J.). Each serum sample was added to two wells containing antigen and to two control wells. Serial dilutions in PBS with 1.5% BSA and Tween 20 were performed with a microtiter plate dilutor. The plates were incubated at 37°C for 1 h and washed three times with PBS-Tween 20 before 125 I-labeled anti-human IgA was added.

The specific binding of VZV IgA antibody was assessed by using goat anti-human IgA specific for the Fc fragment (Tago Inc., Burlingame, Calif.) that had been labeled with 125 I. For use in the RIA, the iodinated anti-IgA was diluted with PBS containing 1.5% BSA and Tween 20 to contain 2×10^6 cpm/ml. The labeled anti-human IgA was added to each well (0.025 ml per well) and incubated for 1 h. The plates were then washed five times with PBS-Tween. Separated wells were counted for 1 min in a gamma scintillation counter.

The controls used in each assay included known positive and negative sera and diluent alone. Specific antibody binding was considered to be present at each dilution for which the ratio of the mean counts per minute for the two wells containing serum and VZV antigen to the mean counts per minute for the two wells containing serum and uninfected tissue control antigen was >2.5 . Background counts per minute in wells containing diluent and VZV antigen were consistently less than 300.

RESULTS

VZV-specific IgA was not detected in any of the 42 subjects who were seronegative for VZV IgG by RIA. VZV-specific IgA was not detected in sera from 20 of the 23 adults who were immune to VZV. Of these 20 sera, 6 contained IgM rheumatoid factor in high titers (1:1,280 to 1:5,120). Titers in the three positive sera were 1:16, 1:64, and 1:1,024. None of these subjects had a known recent exposure to anyone with active VZV infection.

VZV IgA antibody was not detected in sera from 12 VZV IgG-seronegative subjects who had antibody to HSV by immunohemagglutination or from 6 VZV-immune adults who were also HSV seropositive. However, low levels of VZV IgA antibody (1:256) were measured in sera from two of nine VZV-immune subjects who had had recent HSV reactivation.

Serum was obtained within 1 to 5 days after the onset of the varicella exanthem in 36 subjects. The VZV-specific IgA titer was $\geq 1:16$ in 22 of the 36 sera, with a geometric mean titer (GMT) of 1:512. All negative early sera were obtained before day 3 of clinical varicella rash. Twenty-one sera were obtained 5 to 28 days after the onset of rash. All showed VZV IgA titers of $\geq 1:16$, with a GMT of 1:4,674. Five of seven sera obtained more than 28 days after the onset of exanthem had VZV IgA titers of $\geq 1:16$.

FIG. 1. Comparison of the kinetics of the serum IgA, IgG, and IgM responses to primary varicella. Lines connect the dark circles which represent paired sera from an individual subject. (A) Serum IgA; (B) serum IgG; (C) serum IgM. The responses are shown in relation to days after the onset of the varicella exanthem.

The GMT was 1:256. A fourfold or greater rise in titer between acute and convalescent sera occurred in 16 of 22 subjects. The titers in the six subjects who did not exhibit a fourfold rise were initially high and fell fourfold or more in the convalescent specimen. VZV IgA was detected in one of three sera obtained more than 60 days after the onset of the rash.

Figure 1 shows the kinetics of the IgA response compared with IgG and IgM responses for 22 subjects with acute varicella. IgA was detectable in serum as early as was IgG or IgM, but at lower titers.

Five sera were obtained from subjects within 1 week of the onset of herpes zoster. Four of the five sera had detectable VZV-specific IgA antibody, with a GMT of 1:512. All of 10 sera obtained between 1 and 4 weeks after the onset of clinical illness had positive titers, with a GMT of 1:1,024. All sera obtained more than 4 weeks after the onset of rash were positive for VZV

FIG. 2. Serum VZV IgA response after immunization with a live attenuated VZV vaccine. Lines connect the dark circles which represent sequential VZV IgA titers measured in sera from 10 recipients of VZV vaccine over a 25-week period.

IgA, with a GMT of 1:464. A fourfold or greater titer rise was detected in paired sera from five of six subjects. One sustained titer of 1:256 was noted. A VZV IgA titer of 1:1,024 was measured in one subject 63 days after the onset of herpes zoster.

Multiple serum specimens were tested from 10 varicella virus vaccinees (Fig. 2). Serum IgA antibody to VZV was detected in one of three normal adult vaccine recipients. Four of seven children with malignancies who received the vaccine had serum IgA to VZV.

DISCUSSION

The RIA technique proved to be a simple and sensitive method for the measurement of VZV IgA antibodies in unfractionated sera.

IgA antibody to VZV was present very early in the course of varicella in normal subjects. Specific IgA antibody was detected as early as day 2 after the onset of vesicles, and all subjects had VZV IgA by day 3 of exanthem. The pattern of the serum IgA response paralleled VZV-specific IgG and IgM production but the titers of these two antibody classes were severalfold higher than those observed for IgA in this assay (1). Thus, the IgA response to VZV is similar to that observed for other viruses transmitted by the respiratory route. For example, Halonen and associates reported the simultaneous appearance of IgA, IgG, and IgM within 4 days of the onset of the rubella exanthem (11). Peak rubella serum IgA titers were lower than the rubella IgG and IgM titers.

VZV IgA was also produced with the reactivation of latent VZV as documented in subjects with herpes zoster. The GMT of VZV IgA in herpes zoster was higher than the mean VZV IgM titer in this assay, and the IgA titer re-

mained elevated for a longer period than did VZV IgM antibody in normal subjects with herpes zoster (1).

The results obtained when RIA was used to measure VZV IgA production correspond to those obtained by Brunell and associates with an immunofluorescence method (5) and by Levy and Sarov with an enzyme-linked immunosorbent assay (13). Brunell et al. found VZV IgA antibody within 5 days of the appearance of rash and demonstrated that the IgA response was transient. They also detected the appearance of VZV IgA soon after the onset of herpes zoster. By the enzyme-linked immunosorbent assay, the earliest VZV IgA response was detected on day 6 of illness with varicella and on day 5 of illness with herpes zoster. Some subjects with varicella and zoster had no VZV IgA as late as days 10 and 7, respectively. The RIA for VZV IgA has also been useful in documenting a boost in VZV immunity among immune subjects who had household contact with varicella (2). Seventy percent of immune subjects developed detectable VZV IgA with reexposure. Other investigators have found a similar IgA response by immunofluorescent techniques (5). Thus, VZV IgA in serum can be a marker of relatively recent primary or reactivation infection or, in immune subjects, of recent close contact with the virus.

Although the number of subjects tested was small, 5 of 10 recipients of varicella vaccine had detectable serum IgA activity to VZV by RIA, compared with 7% (1/14) of subjects who were tested after subcutaneous vaccination as reported by Bogger-Goren and colleagues, using immunofluorescence (4). These investigators detected secretory IgA in nasal secretions from only 1 of 32 recipients of either inhaled or subcutaneously administered vaccine. It will be of interest to assess the VZV IgA response of vaccinees who have close reexposure to the virus to determine whether these individuals have the same boost in VZV IgA antibody as that seen in naturally immune subjects.

VZV IgA was not detected in sera from any HSV-immune subject who did not have recent clinical herpes, but was detected in sera from 22% (2/9) of immune adults with recent recurrence of HSV infection. This number did not differ significantly from the 13% (3/23) of immune subjects who had measurable VZV IgA titers remote from infection. It is possible that these subjects may have had a recent exposure to VZV with an anamnestic response, or it is possible that in the VZV-immune individual reactivation of HSV can result in a heterologous VZV IgA antibody response that can be detected in this sensitive assay.

Definition of the function of serum IgA in the immune response has been hampered by the

inability to obtain the antibody in pure form until recently. The detection of serum IgA specific for VZV, HSV, cytomegalovirus, and Epstein-Barr virus after an acute infection, which usually involves mucosal surfaces, suggests that IgA plays a role in host defenses against such agents (1, 5, 14, 16).

ACKNOWLEDGMENT

This work was supported by Public Health Service grant AI-17421 from the National Institute of Allergy and Infectious Diseases.

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