Herpes Simplex Virus-Specific Serum Immunoglobulin A: Detection in Patients with Primary or Recurrent Herpes Infections and in Healthy Adults

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A sensitive radioimmunoassay was used to determine levels of herpes simplex virus (HSV)-specific immunoglobulin A (IgA) in serial serum samples drawn from patients with primary HSV infections and from persons with recurrent HSV infections, and in single samples from 90 healthy adults. Significantly rising HSV IgA titers were detected in patients with primary infections, whereas those with recurrent infections had nonfluctuating titers. Sera of IgG-seropositive healthy adults were all positive for HSV-specific IgA without special pretreatment.

It is known that some persons seropositive to herpes simplex virus type 1 (HSV-1) experience recurrent orofacial lesions, whereas others do not (16). Tokumaru suggested (19) that a depression in immunoglobulin A (IgA) antibodies might contribute to reactivation of HSV in subjects with recurrent infection, whereas Nahmias and Roizman have stated that HSV-specific serum IgA is present in some individuals with recurrent infection, but not in others (16). Since it may be that detection of virus-specific serum IgA is highly dependent upon the sensitivity of the technique used, we employed a sensitive radioimmunoassay (RIA), which we have previously used for the detection of serum IgG against several viruses (6, 7, 14), to study the levels of virus-specific IgA in primary and recurrent HSV infection and in healthy adults with and without a history of herpes labialis.

Single serum samples were obtained from 90 healthy medical students and laboratory personnel, and serial serum samples were obtained from seven otherwise healthy adults with recurrent herpes labialis (lesions on the edge of the vermilion border of the lips) and from six patients with recurrent lesions or keratitis from which HSV was isolated. Serial serum samples were also obtained from five patients with stomatitis. Cord sera were obtained from 10 infants of HSV-seropositive mothers.

The RIA procedure used was that described previously for the detection of human IgG directed against varicella (7) or measles virus (6) with the following modifications: HSV antigen was prepared as described by Kimmel et al. (N. Kimmel, M. G. Friedman, and I. Sarov, J. Virol. Methods, in press) and used at a concentration of 200 μg of protein per ml; sera were tested in fourfold dilutions starting from 1:25; the second antibody, labeled with 125I, was rabbit anti-human IgA, obtained from Dakopatts, Copenhagen (no. A092); and titers were determined not by cutoff but as the reciprocal serum dilution at the point where the titration curve on viral antigen reached a distance of one background level (in counts per minute) from the titration curve on control antigen.

Figure 1A shows that for patients with primary herpes infection (stomatitis or seroconversion with respect to HSV-specific IgG, or both), HSV-specific RIA IgA titers rose significantly (greater than fourfold) in the course of the infection.

Figure 1B shows HSV-specific IgA titers determined by RIA for serial serum samples from six patients with recurrent herpes infections (other than labialis). These titers did not fluctuate significantly during the course of the recurrence, although in some cases HSV-specific IgG, determined by immunoperoxidase antibody to membrane antigen (IPAMA) assays (9, 10), did rise significantly. HSV was isolated from each of these patients. Vesicle fluid or corneal scrapings were diluted with Dulbecco modified Eagle medium supplemented with 2% fetal calf serum and were applied to Vero cells grown as described (Kimmel et al., in press). Cytopathic effects were generally detected between 1 and 4 days after inoculation of cell cultures. Infected cells were transferred to glass slides for incubation with a high-titered HSV antiserum, followed by staining by IPAMA for specifically bound IgG.

Figure 1C shows that in serial serum samples from otherwise healthy adults with herpes labialis, HSV-specific IgA titers remained at approxi-
FIG. 1. Comparison of HSV-specific IgA titers determined by RIA in sera of persons with primary versus recurrent herpes infections and in sera of healthy adults. Numbers at the end of solid line curves are patient numbers. (A) Primary herpes infections: These patients had herpetic stomatitis, accompanied in most cases by seroconversion to HSV IgG positive, in all cases with significant rises in HSV-specific IgG titer, as measured by the IPAMA technique (9), and in all cases with HSV-specific IgM detected by enzyme-linked immunosorbent assay (Kimmel et al., in press) in one or more serum samples. Patients 1 to 4 were between 6 and 15 months of age, and patient 5 was 20 years old. (B) Recurrent herpes infections: patients 6, 7, 9, and 11 had herpetic keratitis and ranged in age from 9 months to 71 years. Patients 8 and 10 (9 and 50 years old, respectively) had recurrent extensive lesions (not restricted to the vermilion border of the lips). None of patients 6 through 11 had HSV-specific IgM. HSV was isolated from lesions or corneal scrapings of all these patients. For patient 9, open symbol shows HSV IgA titer (by RIA) for serum sample taken during a recurrent episode of keratitis 3 years previously. (C) Herpes labialis: These patients had herpes lesions restricted to the vermilion border of the lips. All were otherwise healthy. Open symbols show HSV IgA titers of serum samples taken before the current episode of labialis, for persons from whom such samples were available. None of the persons with herpes labialis had HSV-specific IgM in any serum sample when tested by enzyme-linked immunosorbent assay (Kimmel et al., in press). (D) Healthy adults: RIA titers of HSV-specific IgA in serum samples of 47 seropositive healthy adults, 27 with a history of herpes labialis and 20 without. The geometric mean titer (GMT) for each group was 1,260 and 1,580, respectively. The difference in geometric mean titer was borderline with respect to statistical significance ($P = 0.023$), and larger population samples should be examined to ascertain whether the difference may in fact be significant.

When sera of 90 healthy medical students and laboratory personnel were examined, 50 of the healthy adults were seropositive to HSV by IPAMA assay, and in all 50 the RIA detected HSV-specific IgA. Titers ranged from 300 to 6,400 with a geometric mean titer of 1,360. Of the 50 seropositive adults, 27 stated that they were subject to recurrent herpes labialis and 20 stated that they were not (3 could not be contacted). Figure 1D shows the HSV-specific serum IgA titers of the subjects in these two groups. The geometric mean titer ($\log_{10}$) of the group subject to herpes labialis was $3.1 \pm 0.4$ (titer, 1,260), and that of the second group was $3.2 \pm 0.3$ (titer, 1,580). A comparison of Fig. 1B, C, and D demonstrates that these titers are in the same range as those found for persons with ongoing recurrent herpes infections. All 40 seronegative adults did not have HSV IgA by RIA.

To verify that the RIA technique was detecting IgA and not IgG, we examined 10 cord serum samples from infants of HSV-seropositive mothers. No HSV-specific IgA was detected in any of the 10 serum samples (titer, <25).

To rule out the possibility that we were detecting an IgA-class rheumatoid factor, we pretreated sera either with aggregated IgG, prepared according to the method of McCarthy et al. (15), or by staphylococcal absorption (8). Neither treatment resulted in a significant change in IgA
titer (fourfold or greater). In contrast to the "unmasking" effect of protein A described by Ratner et al. (18), all 90 seropositive healthy adults whom we tested were positive for HSV-specific IgA without staphylococcal absorption of the sera. The differing results may be due to different relative amounts of viral antigen available to react with serum antibodies in the two RIA techniques.

With regard to serum IgA titers found in seropositive healthy adults and persons with recurrent infections, El Falaky et al. reported elevated titers of HSV-specific serum IgA (by immunofluorescence) in persons with herpes infections as compared to control seropositive healthy adults (5). However, they did not distinguish between primary and recurrent herpetic infections, and they used a different antigen (whole dried and fixed cells) from ours so that it is difficult to compare directly our results and theirs.

Our preliminary results showing comparable HSV-specific RIA IgA titers for 47 seropositive healthy adults (27 with and 20 without recurrent herpes labialis; Fig. 1D) support the suggestions of Centifanto and co-workers (3), based on their rabbit experiments, and of Douglas and Couch (4) that a reduced level of virus-specific IgA is probably not responsible for recurrence of lesions. Zweerink and Stanton found by a radioimmunoprecipitation assay that recurrent herpetic infections could not be correlated with quantitative or qualitative changes in the levels of HSV-specific IgG antibodies directed against individual polypeptides (20). Similar studies should be carried out with respect to viral antigen-specific IgA antibodies in serum and secretions to establish firmly whether fluctuations in IgA antibodies are associated with recurrent HSV infections.

The fact that we found HSV-specific IgA in all seropositive individuals by RIA whereas others have reported that it is not always present (16) implies that detection of this antibody may depend upon the technique used; likewise the reported disappearance of virus-specific IgA from the serum after recovery from various viral illnesses (1, 12, 17) should be reexamined. For example, Halonen et al., with a sensitive RIA, detected IgA antibodies to respiratory syncytial virus in all sera of a series taken from patients with adenovirus infections (11).

It will be interesting to learn whether persistence of virus-specific IgA is a general phenomenon, the detection of which is dependent only upon the sensitivity of the technique used, or whether continued antigenic stimulation is required. If the latter is true, then for HSV it may be that the immune system receives a certain amount of viral antigenic stimulation regardless of whether there is a clinical recurrence or not, since it has been reported (2, 13) that HSV can sometimes be isolated from secretions of persons without obvious lesions.

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LITERATURE CITED
