

Differentiation of Primary from Nonprimary Genital Herpes Infections by a Herpes Simplex Virus-Specific Immunoglobulin G Avidity Assay

MADOKA HASHIDO,^{1*} SAKAE INOUE,¹ AND TAKASHI KAWANA²

Department of Epidemiology, National Institute of Health,¹ and Department of Obstetrics & Gynecology, Tokyo University Branch Hospital,² Tokyo, Japan

Received 2 December 1996/Returned for modification 7 January 1997/Accepted 14 April 1997

An immunoglobulin G (IgG) antibody avidity assay which uses protein-denaturing agents and a modification of an enzyme-linked immunosorbent assay have been investigated for their usefulness in distinguishing primary genital herpes simplex virus (HSV) infections from nonprimary infections. Forty-nine serum specimens from patients with primary, recurrent, and nonprimary first-episode genital herpes were studied. The clearest separation was obtained with 6 M urea treatment, giving mean avidity indices of 0.398 for sera ≤ 100 days after the infection and 0.879 for sera > 100 days after the infection ($P < 0.001$). No significant difference in avidity indices was observed between the recurrent and nonprimary first-episode infections. Determination of the avidity of HSV-specific IgG will improve the diagnostic potential of HSV serology.

Although herpes simplex virus (HSV) infections are usually asymptomatic or associated with a localized illness, severe, life-threatening disease can follow in newborn infants or immunocompromised hosts, including human immunodeficiency virus-infected individuals, with involvement of the central nervous system (17). Several studies have revealed that the major risk factor to the fetus or newborn infants is maternal primary genital HSV infection (19, 25). However, 70 to 80% of primary HSV infections are asymptomatic (17), and even with symptomatic manifestations, primary infections cannot be clinically differentiated from nonprimary infections (14). Thus, serological tests serve as a useful adjunct not only for epidemiological and etiological studies but also for clinical studies to elucidate HSV infections.

Generally, in the diagnosis of viral diseases, confirmation of primary infections depends on the detection of immunoglobulin M (IgM) or IgG seroconversion comparing the titers of acute- and convalescent-phase serum samples. However, well-timed collection of paired sera is often an impractical requirement. Furthermore, in HSV infection, the IgM response is not unique to the primary phase, but the persistence or reappearance of IgM is often observed during recurrences or even in subjects without clinical symptoms (7, 20). To overcome these difficulties, a unique serodiagnostic approach which differentiates primary HSV infections by the characteristic presence of the polymeric form of specific IgA antibody has been developed (8). This technique, which requires sedimentation and fractionation procedures with a supercentrifuge, however, still could not be regarded as the most practical method in clinical laboratories.

Recently, it has been demonstrated that the early specific IgG response after primary infections consists of low-avidity antibodies, with maturation of avidity in a few months (23). Low-avidity-antibody denaturation techniques have been applied effectively to the diagnosis of a variety of human infectious diseases including rubella (5, 9, 11, 18, 21), varicella-

zoster virus, (12, 13), cytomegalovirus (1, 2), Epstein-Barr virus (4, 6), human herpesvirus 6 (22), and toxoplasma infections (10).

In this study, to evaluate the diagnostic value of measuring the avidity of HSV-specific IgG antibody, we investigated the IgG avidity following genital HSV infections using 6 M urea, 8 M urea, and 35 mM diethylamine (DEA) as denaturing agents. We confirmed that IgG avidity can serve as a serological marker in the differentiation of primary from recurrent or nonprimary first-episode genital herpes infections.

MATERIALS AND METHODS

Forty-nine serum samples were collected from female genital herpes patients attending the department of Obstetrics and Gynecology, Tokyo University Branch Hospital. Diagnosis was made on the basis of clinical symptoms and viral culture (14). Isolated viruses were identified by fluorescein isothiocyanate-labeled monoclonal antibodies against HSV type 1 (HSV-1) and HSV-2 (Micro-Trak, Syva, Calif.). On the basis of clinical history and neutralizing antibody response, these cases of genital herpes were classified into three types: the primary type (HSV-1, 9 cases; HSV-2, 3 cases), the recurrent type (HSV-1, 8 cases; HSV-2, 19 cases), and the nonprimary first-episode type (HSV-1, 4 cases; HSV-2, 6 cases). The primary type was diagnosed if the HSV neutralizing antibody titer in the acute-phase serum was < 8 . If the titer was ≥ 8 , without a significant increase in the convalescent-phase serum, the patient was considered to have a recurrent or nonprimary first-episode infection. With a previous history of genital herpes, the patient was diagnosed as having a recurrent type of infection. If there was no previous history, the patient was diagnosed as having a nonprimary first-episode infection.

The HSV IgG enzyme-linked immunosorbent assay (ELISA) was performed as reported previously (8). In brief, adequately diluted Nonidet P-40-lysed extracts of HEL-R66 cells infected with HSV-1 (TVK-171; a clinical isolate) or HSV-2 (THH-54; a clinical isolate) were coated onto microplates in 0.1 M carbonate buffer (pH 9.6). Mock-infected cell extracts were used as a control antigen. A 100- μ l volume of serum specimen diluted adequately, affinity-purified goat F(ab')₂ to human IgG labeled with horseradish peroxidase (Tago Inc., Burlingame, Calif.) diluted 1:2,000, and the substrate *ortho*-phenylenediamine in 0.1 M citrate-phosphate buffer were successively added to the plates, and the plates were incubated at room temperature for 90, 60, and 20 min for these steps, respectively. The dilution of serum specimen was predetermined to finally produce an optical density (OD) value within the quantitative range on the dose-response curve, and each serum specimen was applied to a type of HSV antigen homologous to that of the isolated virus. The HSV-specific IgG antibody activity was calculated as (OD of the well coated with HSV antigen) - (OD of the well coated with control antigen).

HSV-specific IgG avidity was measured with protein-denaturing agents. Quadruplicate microplates were washed with phosphate-buffered saline (PBS) and 6 M urea-, 8 M urea-, or 35 mM DEA-containing PBS solution. A 150- μ l volume of the elution agent or PBS was added to each well following incubation of the serum specimen in antigen-coated plates. After an 8-min exposure to the agent at room temperature, the plates were washed and processed as described above.

* Corresponding author. Mailing address: Department of Epidemiology, National Institute of Health, Toyama 1-23-1, Shinjuku-ku, Tokyo 162, Japan. Phone: 81-3-5285-1111, ext. 2556. Fax: 81-3-5285-1129. E-mail: mdkhsd@nih.go.jp.

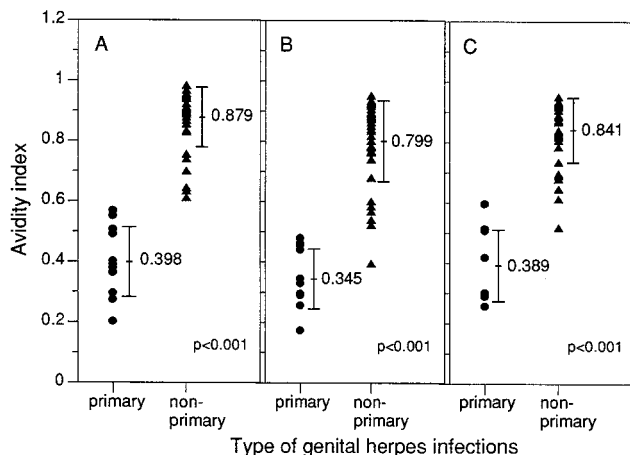


FIG. 1. AIs of HSV-specific IgG antibody in patients with primary and non-primary infections: comparison of 6 M urea, 8 M urea, and 35 mM DEA as elution agents. (A) Treatment with 6 M urea. (B) Treatment with 8 M urea. (C) Treatment with 35 mM DEA.

HSV-specific IgG antibody activities in the wells washed with the elution agents or PBS only were used to calculate the avidity index (AI), where AI = (HSV-specific IgG antibody activity of the wells washed with an elution agent)/(that of the wells washed with PBS).

Student's *t* test was used to estimate the significance of the difference between the mean values.

RESULTS

Comparison of eluting efficiencies among 6 M urea, 8 M urea, and 35 mM DEA in differentiation of primary and non-primary HSV infections. The AI for the sera from patients with primary infection (≤ 100 days after the onset of disease) and nonprimary infection were obtained by ELISA with 6 M urea, 8 M urea, or 35 mM DEA as the elution agents (Fig. 1). Significant differences in AI were observed between the groups with primary and nonprimary infections by all three agents ($P < 0.001$). However, the sera treated with 8 M urea or 35 mM DEA showed overlapping of the AIs between the groups with primary and nonprimary infections. The clearest separation of the AI between two groups was achieved by using 6 M urea as an elution agent. For the further studies, treatment with 6 M urea was used as a method of choice.

Relationship between HSV-specific IgG avidity and time after infection. The AIs of 20 serum specimens taken at various times after the primary infection are presented in Fig. 2. The AI markedly increased during the first 100 days after the infection. The mean \pm standard deviation AIs were 0.363 ± 0.099 during the first 50 days and 0.509 ± 0.084 between 50 and 100 days. All the sera had AIs of ≥ 0.6 (0.884 ± 0.085) after 100 days.

HSV-specific IgG avidity in primary, recurrent, and nonprimary first-episode genital herpes. The AIs of sera taken from patients with primary genital herpes were significantly lower than those from patients with recurrent or nonprimary first-episode types for patients with either HSV-1 or HSV-2 infections (Fig. 3). The difference in AIs between patients with recurrent and nonprimary first-episode infections was not significant ($P > 0.05$), although the sera collected from patients with recurrent infections tended to give a wider range of AIs, with lower mean values. Differences in the numbers and sampling times of the serum specimens between patients with primary HSV-1 infection and those with primary HSV-2 infection caused variance in the distribution of AIs, but with no statistical significance.

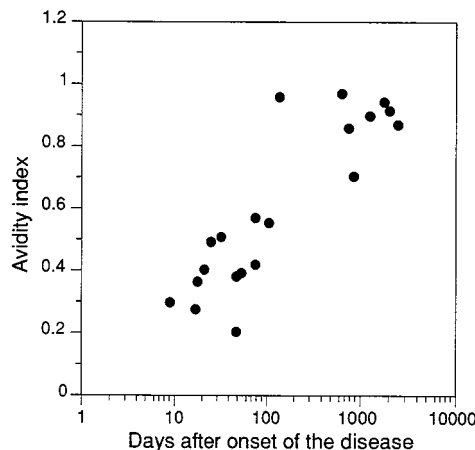


FIG. 2. Maturation of HSV-specific IgG antibody avidity in relation to time after the primary infection.

DISCUSSION

This study showed that the humoral response following HSV infection shows the expected time-dependent increase in antibody avidity. Although other workers have successfully used 8 M urea or 35 mM DEA for other pathogens, we found 6 M urea to be a suitable elution agent for measuring the avidity of HSV-specific IgG. The 6 M urea treatment showed the clearest separation between the sera collected from patients with primary and nonprimary infections, whereas the use of 8 M urea or DEA resulted in overlapping AI values, making the former the method of choice.

We have confirmed the findings reported by others that the primary and nonprimary infections can be separated by the presence of specific IgG of low and high avidities (1, 2, 4-6, 9-13, 18, 21, 22) and showed that the avidity of HSV-specific IgG increases up to 100 days after infection before reaching a plateau. As reported for cytomegalovirus (1, 2), Epstein-Barr virus (4, 6), human herpesvirus 6 (22), and varicella-zoster virus (12, 13) infections, the procedure described above is particularly beneficial in the diagnosis of chronic infectious diseases in which detection of the rise of IgM or IgG antibody titers has little practical value (7, 20).

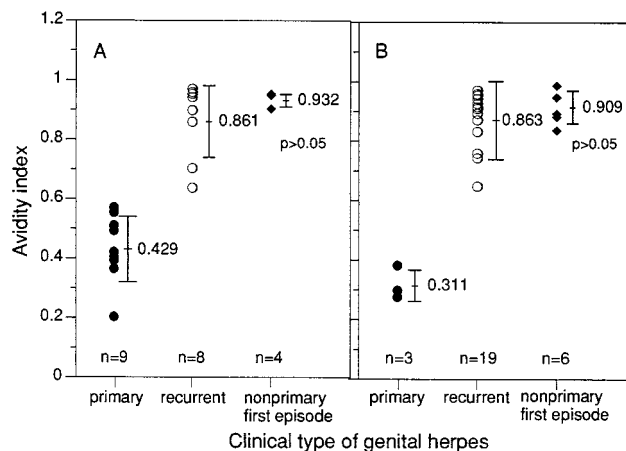


FIG. 3. AIs of HSV-specific IgG antibody in patients with primary, recurrent, and nonprimary first-episode genital herpes. (A) HSV-1-infected patients (B) HSV-2-infected patients.

The information whether the patient had primary HSV infection during the previous 100 days can help the clinician from epidemiological and etiological viewpoints, but it can also help clinically.

First, the information presented above is important for studying the epidemiology and the natural history of HSV infections. Because most primary HSV infections are asymptomatic and even with symptomatic manifestations primary infections cannot be clinically differentiated from nonprimary infections, the serodiagnostic approach is indispensable in understanding HSV infections.

Second, the information presented above may play an important role in elucidating the pathogenesis of HSV-related diseases, such as encephalitis or keratitis, for which the risk factors have not yet been identified (17, 24).

Third, in cases of neonatal herpes, serological identification of primary HSV infections during the previous 100 days in the mothers gives an important clue to the clinician who is interested in tracing the source and time of infection for his or her patients. As reviewed well by Whitley (25) and Randolph et al. (19), maternal primary HSV infection is the greatest risk factor for fetal or neonatal herpes, resulting in more serious consequences than nonprimary infections in the rate of vertical transmission (50 versus 1%) and the severity of disease in the infected child. However, about 70% of the mothers who gave birth to an infected child had never noticed symptoms of genital herpes (3), and thus needed serological assessment.

Also, Fitzgerald et al. (5) and Boppana and Britt (2) suggested that the affinity maturation of maternal antibody may be critical in preventing maternal-fetal transmission of rubella or cytomegalovirus. In their reports, nontransmitters showed significantly higher antibody avidities than transmitters. We expect that the measurement of antibody avidity in HSV-infected pregnant women will be useful for predicting the risk factors for fetal or neonatal HSV infections.

In our study, the sera collected from patients with recurrent infection tended to give a wider range of AIs, with lower mean values than those from patients with nonprimary first-episode infection, although the difference was not significant. The higher AIs observed for sera from patients with recurrent and nonprimary first episodes of HSV-2 infection may be explained by the influence of an anamnestic reaction, i.e., the influence of cross-reactive antibody avidity against HSV-1 which had infected those patients previously. HSV type-specific antibody analysis (15, 16) revealed the presence of the HSV-1 antibody in addition to the HSV-2 antibody in 7 of the 19 patients with recurrent HSV-2 infection and in all 6 patients with nonprimary first episodes of HSV-2 infection (data not shown). The interference of the avidity result by the anamnestic effects is under investigation in our laboratory in a comparison of homologous and heterologous types of HSV antigens.

The lower AIs for patients with recurrent HSV-1 and HSV-2 infections also may be explained by the immunological or genetic factors of the hosts. Junker and Tilley (12) studied a group of apparently immunocompetent children who had had multiple episodes of chicken pox and could not find a high-avidity antibody characteristic of the anamnestic response at the time of the repeated chicken pox episodes. An inability to select high-affinity HSV antibodies may be associated with frequent recurrences of symptoms, although in this study, related clinical information could not be acquired for those patients.

In conclusion, determination of the avidity of HSV-specific IgG will be useful for improving the significance and diagnostic potential of HSV serology, especially without a requirement for adequate sampling of acute-phase sera.

ACKNOWLEDGMENT

This work was performed at the department of Epidemiology, National Institute of Health, Tokyo, Japan.

REFERENCES

- Blackburn, N. K., B. D. Besselaar, B. D. Schoub, and K. F. O'Connell. 1991. Differentiation of primary cytomegalovirus infection from reactivation using the urea denaturation test for measuring antibody avidity. *J. Med. Virol.* **33**:6-9.
- Boppana, S. B., and W. J. Britt. 1995. Antiviral antibody responses and intrauterine transmission after primary maternal cytomegalovirus infection. *J. Infect. Dis.* **171**:1115-1121.
- Brown, Z. A., J. Benedetti, S. Selke, R. Ashley, D. H. Watts, and L. Corey. 1996. Asymptomatic maternal shedding of herpes simplex virus at the onset of labor: relationship to preterm labor. *Obstet. Gynecol.* **87**:483-488.
- De Ory, F., J. Antonaya, M. V. Fernandez, and J. M. Echevarria. 1993. Application of low-avidity immunoglobulin G studies to diagnosis of Epstein-Barr virus infectious mononucleosis. *J. Clin. Microbiol.* **31**:1669-1671.
- Fitzgerald, M. G., G. R. Pullen, and C. S. Hosking. 1988. Low affinity antibody to rubella antigen in patients after rubella infection in utero. *Pediatrics* **81**:812-814.
- Gray, J. J. 1995. Avidity of EBV VCA-specific IgG antibodies: distinction between recent primary infection, past infection and reactivation. *J. Virol. Methods* **52**:95-104.
- Hashido, M., and T. Kawana. Herpes simplex virus-specific IgM, IgA, and IgG subclass antibody responses in primary and nonprimary genital herpes patients. *Microbiol. Immunol.*, in press.
- Hashido, M., T. Kawana, and S. Inouye. 1989. Differentiation of primary from nonprimary genital herpes simplex virus infections by detection of polymeric immunoglobulin A activity. *J. Clin. Microbiol.* **27**:2609-2611.
- Hedman, K., and I. Seppala. 1988. Recent rubella virus infection indicated by a low avidity of specific IgG. *J. Clin. Immunol.* **8**:214-221.
- Holliman, R. E., R. Raymond, N. Renton, and J. D. Johnson. 1994. The diagnosis of toxoplasmosis using IgG avidity. *Epidemiol. Infect.* **112**:399-408.
- Inouye, S., A. Hasegawa, S. Matsuno, and S. Katow. 1984. Changes in antibody avidity after virus infections: detection by an immunosorbent assay in which a mild protein-denaturing agent is employed. *J. Clin. Microbiol.* **20**:525-529.
- Junker, A. K., and P. Tilley. 1994. Varicella-zoster virus antibody avidity and IgG-subclass patterns in children with recurrent chickenpox. *J. Med. Virol.* **43**:119-124.
- Kangro, H. O., S. Manzoor, and D. R. Harper. 1991. Antibody avidity following varicella-zoster virus infections. *J. Med. Virol.* **33**:100-105.
- Kawana, T., K. Kawagoe, K. Takizawa, J. T. Cheng, T. Kawaguchi, and S. Sakamoto. 1982. Clinical and virologic studies on female genital herpes. *Obstet. Gynecol.* **60**:456-461.
- Lee, F. K., R. M. Coleman, L. Pereira, P. D. Bailey, M. Tatsuno, and A. J. Nahmias. 1985. Detection of herpes simplex virus type 2-specific antibody with glycoprotein G. *J. Clin. Microbiol.* **22**:641-644.
- Lee, F. K., L. Pereira, C. Griffin, E. Reid, and A. J. Nahmias. 1986. A novel glycoprotein for detection of herpes simplex type 1-specific antibodies. *J. Virol. Methods* **14**:111-118.
- Nahmias, A. J., H. Keyserling, and F. K. Lee. 1989. Herpes simplex viruses 1 and 2, p. 393-417. *In* A. S. Evans (ed.), *Viral infections of humans*. Plenum Press, New York, N.Y.
- Pullen, G. R., M. G. Fitzgerald, and C. S. Hosking. 1986. Antibody avidity determination by ELISA using thiocyanate elution. *J. Immunol. Methods* **86**:83-87.
- Randolph, A. G., A. E. Washington, and C. G. Prober. 1993. Cesarean delivery for women presenting with genital herpes lesions. Efficacy, risks, and costs. *JAMA* **270**:77-82.
- Shore, S. L., and A. J. Nahmias. 1982. Immunology of herpes simplex viruses, p. 21-72. *In* A. J. Nahmias and R. J. O'Reilly (ed.), *Immunology of human infection*. Part II. Plenum Press, New York, N.Y.
- Thomas, H. I. J., and P. Morgan-Chapner. 1988. Rubella-specific IgG subclass avidity ELISA and its role in the differentiation between primary rubella and rubella reinfection. *Epidemiol. Infect.* **101**:591-598.
- Ward, K. N., J. J. Gray, M. W. Fotheringham, and M. J. Sheldon. 1993. IgG antibodies to human herpesvirus-6 in young children: changes in avidity of antibody correlate with time after infection. *J. Med. Virol.* **39**:131-138.
- Werblin, T. P., Y. T. Kim, F. Quagliata, and G. W. Siskind. 1973. Studies in the control of antibody synthesis. III. Changes in the heterogeneity of antibody affinity during the course of the immune response. *Immunology* **24**:477-492.
- Whitley, R. J. 1996. Herpes simplex virus, p. 2297-2342. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), *Fields virology*. Lippincott-Raven Publishers, Philadelphia, Pa.
- Whitley, R. J. 1994. Herpes simplex virus infections of women and their offspring: implications for a developed society. *Proc. Natl. Acad. Sci. USA* **91**:2441-2447.