Diseases caused by herpes simplex virus (HSV) range from mild illness, indiscernible in the majority of patients, to sporadic, severe and life-threatening disease in newborn infants or immunocompromised hosts (35). Although two distinct serotypes of HSV-1 and HSV-2 are usually transmitted by different routes and involve different areas of the body, the epidemiology and clinical manifestations of infections overlap considerably (27).

Genital herpes has been a serious public health problem, generating a large pool of individuals who are potentially infectious, while putting the sufferers under great stress physically and/or psychologically. Since only one-third, at most, of genitally infected individuals are symptomatic (22), serologic tests serve as an indispensable adjunct not only for diagnosis but also for clinical and epidemiological studies. However, the unique characteristics of HSV, such as the establishment of latency, reactivation and reinfection with HSV-2 over HSV-1, have made the serologic assessment of genital herpes substantially difficult.

In spite of a variety of studies on humoral immunity in HSV infections (5, 7, 9, 20, 24, 26, 30, 32), incorporative studies are lacking on distinct clinical features of the disease with a sufficient follow-up of patients on the changes in antibody response under different conditions. Thus, the details on humoral immunity in genital HSV infections remains unknown.

We analyzed IgM, IgA and IgG subclass-specific antibody responses against HSV in serum samples collected serially from patients with primary, recurrent and nonprimary first episode types of genital herpes. By developing an enzyme-linked immunosorbent assay (ELISA), we obtained new findings on the characteristic patterns of HSV-specific IgM, IgA and IgG4 antibody responses in primary and nonprimary infections, giving useful information for interpreting the HSV serology.

Materials and Methods

Subjects and serum specimen. The subjects were 36 female genital herpes patients attending the Department of Obstetrics and Gynecology, Tokyo University Hospital between 1972 and 1985. Genital herpes was diagnosed...
statistical analysis. The χ² test with Yate's correction was used to test the differences in antibody detection rates.

Results

HSV-Specific Antibody Responses in Primary Genital Herpes

The serial change of HSV-specific antibody activities over time after onset of the disease in primary genital herpes is shown in Fig. 1, A and B. In most cases IgM and IgA showed peaks within 2–3 weeks after onset and then decreased, while IgG1 and IgG3 tended to increase. For HSV-2 infection, IgG1 and IgG3 tended to increase more rapidly than in HSV-1 infection. Although some variations were observed among the patients on the pattern of each antibody response, no related information was available from their clinical records. IgM, IgA, IgG1 and IgG3 antibodies were detected at a rate of 100%, either in HSV-1- or HSV-2-isolated cases (Table 1). As IgG2 was not detected in any case of HSV-1 or HSV-2 primary infection, its response is not described in Fig. 1. IgG4 was detected only in 1 case of HSV-1 infection on day 489 and in 1 case of HSV-2 infection on day 264 (data not shown).

HSV-Specific Antibody Responses in Recurrent Genital Herpes

In recurrent genital herpes, IgA, IgG1 and IgG3 antibodies were detected in all sera obtained during recurrence, whereas IgM was detected in none (0%) of the 3 HSV-1-isolated cases and in 8 of the 10 (80%) HSV-2-isolated cases (Table 1). IgG4 was detected in all 3 (100%) HSV-1 cases and in 7 of the 10 (70%) HSV-2 cases. IgG2 was not detected in any case. The serial change of HSV-specific antibody activities over time after onset of the disease in recurrent genital herpes is shown in Fig. 2, A and B.

HSV-Specific Antibody Responses in Nonprimary First Episode Genital Herpes

Similarly to the recurrent cases, IgA, IgG1 and IgG3
Table 1. Number of patients positive for HSV-specific antibodies: comparison between primary, recurrent and nonprimary first episode genital herpes

| Clinical type of genital herpes | HSV type | Number of patients | Number of antibody-positive cases
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<td>IgM</td>
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<td>Recurrent</td>
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<td>Nonprimary first episode</td>
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*Number of patients in whom the antibody was detected within 30 days after onset of the disease.

Fig. 1. HSV-specific IgM, IgA, IgG1 and IgG3 antibody responses in primary genital HSV infections. A. Primary infections by HSV-1. B. Primary infections by HSV-2. -----, cut-off lines.

Fig. 2. HSV-specific IgM, IgA, IgG1, IgG3 and IgG4 antibody responses in nonprimary genital HSV infections. A. Recurrent infections by HSV-1. B. Recurrent infections by HSV-2. C. Nonprimary first episode infections by HSV-2. -----, cut-off lines.
antibodies were detected in all sera including those from the acute phase (Table 1), and did not fluctuate significantly in nonprimary first episode genital herpes. IgM and IgG4 were detected in 5 out of 6 (83%) and in 4 out of 6 (67%) cases, respectively. IgG2 was not detected in any case. The serial change of HSV-specific antibody activities over time after onset of the disease in nonprimary first episode genital herpes is shown in Fig. 2C. The levels of antibody activity of IgM, IgG1 and IgG3 tended to be lower in the nonprimary first episode cases than in the recurrent cases.

**Discussion**

We analyzed IgM, IgA and IgG subclass-specific antibody responses against HSV in the serial sera taken from genital herpes patients and found several characteristic patterns in primary and nonprimary infections.

First, we confirmed earlier reports (20, 29) that IgM was detected not only in primary but also in nonprimary infections, the rate being as high as 68% (13/19). Also, we noticed that in those nonprimary cases, IgM did not show the distinct peak seen in the primary infection. In addition to our previous data that 18% of HSV IgG-seropositive subjects without clinical symptoms had anti HSV-IgM (14), the above data clearly suggests that IgM cannot be used as a diagnostic marker for primary HSV infection.

IgA also showed a unique pattern. In primary infection, IgA gave a similar but sharper peak than IgM in most cases. During nonprimary episodes it was detected in relatively high levels. As we demonstrated previously using a supercentrifuge-fractionation technique (13), IgA which increases shortly after the primary infection is polymeric IgA, whereas the IgA detected stably in nonprimary infections is monomeric IgA. It is of interest to explain the presence of a marked peak of polymeric IgA after the primary infection by the concept of common mucosal immunity (6); that is, the precursors of IgA plasma cells arising in one mucosal area may home in on another mucosal area as well as travel back to the initial area through the bloodstream. This means that, during this homing, IgA plasma cells committed to HSV may secret polymeric IgA into the bloodstream.

Human IgG antibodies consist of 4 subclasses, IgG1, IgG2, IgG3 and IgG4, at 66, 23, 7 and 4%, respectively (36). However, the antibody response to a particular antigen is not in agreement with these amounts. For example, to carbohydrate antigens, IgG2 was presented to be a dominant responder (3), while in parasitic infections (1, 10, 12, 28) or allergic reactions (15) where chronic stimulation by antigen takes place, IgG4 may play some role. In viral infections such as rotavirus (11), respiratory syncytial virus (34), measles (25), mumps (29), HIV (31), varicella-zoster virus (7, 8) and cytomegalovirus infections (7, 17, 33), IgG1 and IgG3 were prominent, and IgG2 and IgG4 were never or rarely detected.

In this HSV study, mainly IgG1 and IgG3 were detected in both primary and nonprimary infections, and IgG2 was never detected in any case. Since Coleman et al (5) indicated that the sensitivity of an assay was highly dependent on the clone of MoAb used as a detector, we carefully selected the clones on which WHO/UI/S had confirmed sensitivity and specificity in the ELISA system (16), and furthermore, we pre-evaluated the competency of those MoAbs using human IgG subclass myeloma proteins (data not shown). It is hard to estimate that the non-detectability of IgG2 is directly associated with the antigen composition, since we confirmed, by Western blot, that the antigens used here contain most of the major HSV immunogenic proteins, such as glycoprotein D (gD), gB, gC, gE and gG, by use of MoAbs against them. We also showed that the protein bands of the antigens electrophoresed were broadly reactive with multiple HSV-immune human sera, indicating the presence of many HSV-induced proteins (data not shown). We cannot, however, exclude the possibility that the anti-IgG2 MoAb HP6014 we used might be unable to recognize its targeted epitope due to conformational difficulties caused inherently in our ELISA system.

IgG4 was not detected until at least 9 months after the primary infection, but was highly detectable in the nonprimary cases in our study. A finding by Hagan et al (12) that, in Schistosoma haematobium infection, IgG4 recognized antigens in the same pattern as IgE did, together with a finding by Legace-Simar et al (23) that, in patients with frequently recurrent genital herpes, the level of total IgE was elevated, suggests that repeated antigen stimulation by the reactivation of HSV lead to the production of IgG4 antibody.

In this study, some variations were observed among the patients on the pattern of antibody response. However, partly due to the small number of cases and the lack of related clinical information, no clear association was noticed between the antibody response and clinical manifestations or prognosis of the disease. In general, no significant difference was observed in the antibody responses between the recurrent and nonprimary first episode types of genital herpes, nor between HSV-1 and HSV-2 infection. It is interesting that IgG1 and IgG3 showed a more rapid increase in HSV-2 primary infection than in HSV-1. It might suggest the possibility of anamnesic reaction by previously-infected HSV-1 which generated no detectable level of antibody before the episode. This should be of concern for future investigation. The
tendency of IgM, IgG1 and IgG3 antibody levels to be higher in recurrent cases than in nonprimary first episode cases may reflect more frequent recrudescence or reactivation of latent viruses in recurrent cases.

Several reports including ours revealed that acyclovir treatment suppresses the production of HSV-specific antibody and lowers the antibody responses, especially that of IgM, in primary HSV infection (2, 4, 18). Therefore, it is noteworthy that the sera used here were collected during 1972 to 1985 from the patients under no antiviral treatment, and that the results obtained describe the natural course of the humoral immune response in genital HSV infection, although the number of cases examined was small.

In conclusion, useful findings were obtained regarding the antibody response in genital HSV infections: 1) the detection of IgM in both primary and nonprimary infections, 2) a sharp peak of IgA in primary infection, and 3) the detection of IgG4 only in nonprimary infection, which may improve the diagnostic potential of HSV serology.

References

2 herpes simplex virus infection among women. STD 17: 90–94.